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REGARDING THE EXISTENCE OF THE 'COMMON CHEMICAL SENSE' IN VERTEBRATES¹

W. J. CROZIER

I. In a recent paper by Coghill ('14) exception is taken to the view held by Herrick ('08), Sheldon ('09), Cole ('10), and Parker ('12), that there is in vertebrates a set of receptors (free nerve terminations) which are responsible for reactions to rather high concentrations of chemicals when applied to moist peripheral surfaces. The theoretical significance attached to this 'common chemical sense' (Parker, '12) makes it appropriate to bring forward certain facts which may to some extent serve to clear up the situation.

The evidence adduced by Coghill is this: that in *Amblystoma* larvae, before the establishment of the definitive nervous system, tactile and chemical irritability appear concomitantly and, so far as studied, remain completely parallel in development; and that if larvae be placed in irritating solutions of hydrochloric acid (as dilute as $\frac{N}{10,000}$), the epithelial cells become visibly disrupted, the reaction of the larvae being correlated with the disintegration of the skin and a gradual disappearance of reaction to tactile stimuli.

His conclusion, that there is not present in the skin any normal irritability to acid, is probably correct for the larvae experimented upon; but it by no means follows that the same conditions obtain in the adult. Coghill argues, however, that the permanently embryonic cells of the germinative layer in the skin of amphibians and fishes when exposed to the high concentrations of acid ($\frac{N}{40}$) used by Parker and Sheldon act as do the superficial ciliated cells of the *Amblystoma* larval skin. Because they are bound down by a "thick, less sensitive, and

¹ Contributions from the Bermuda Biological Station for Research. No. 40.

more impervious layer of cells," the disintegration of the sensitive cells would cause exceedingly violent disturbances, presumably affecting tactile and pain terminals; and further, that it has not been satisfactorily demonstrated that it is possible (as claimed by Sheldon and by Cole) to effect a separation of tactile and 'common chemical' irritability in fishes and amphibians.

This general argument is somewhat strengthened by the fact (to which Coghill does not refer) that aquatic vertebrates possessing a soft slimy skin—cyclostomes, eels, catfish, *Necturus*—are known to react to local irritation by chloroform and other substances by the expulsion of mucus and even of entire gland cells. The reaction time of this response is not known accurately, though it is judged to be much greater than that, for example, of the catfish as a whole when stimulated by hydrochloric acid applied to its trunk region; still, it is not inconceivable that mechanical deformations brought about in this way might provide stimuli for tactile nerve endings, provided the mucus response were sufficiently sharp and prompt.

II. The exact manner in which solutions are to produce the hypothesized effects upon the cells of the germinative layer is left entirely untouched by Coghill. The rapidity of the responses given by the catfish and *Necturus* immediately negatives the idea that osmotic transfer of water is the agency of stimulation. In the case of the spinal frog, as studied by Braeuning ('04), Loeb ('05), and Cole ('10), though the reaction times are rather long, there is abundant evidence that other than osmotic factors are at work. The most important point which arises for consideration is the extent to which chemical stimulants actually penetrate the skin, i.e., the degree to which the cells of the germinative layer are exposed to the action of the stimulant. In addition to the rapidity of the reactions under discussion, it is to be remembered that they are excited by acids, salts, alcohols, and a variety of other substances.

That the skin of aquatic animals is not to any appreciable degree damaged by the agents responsible for 'common-chemical-sense' reactions, is clearly indicated by such facts as the following:

1. High concentrations of acids ($\frac{N}{50}$), salts (2N), alkalies ($\frac{N}{50}$) and alkaloids ($\frac{M}{100}$), which evoke prompt and vigorous reactions from the earthworm, *Eisenia foetida*, cease to cause any disturbance the moment the stimulant is washed off by immersing the animal in water. If any serious disintegration were produced by these solutions, it would be reasonable to expect the continuance of activity after the external supply of the stimulant had been removed.²

2. Holothurians, such as *H. surinamensis* (Crozier, '15 c) and *Stichopus moebii* (Crozier, '15 b), possess skin pigments whose loss is a fairly good indicator of changes in permeability. Yet in no case on the application, by the pipette method, of the stimuli used by Parker ('12) was there any indication of pigment loss accompanying reaction.³ This is true also of the nudibranch, *Chromodoris zebra*.

III. The value of the intracellular indicator of *Chromodoris* in the study of cell penetration by acids has been pointed out elsewhere (Crozier, '15 b). When small volumes of acids, even in $\frac{N}{10}$ solution, are used to stimulate *Chromodoris*, there is no visible evidence that they penetrate the skin. In fact the time required for the penetration of the acids is quite high (see also Harvey, '14), the most rapid rate observed being with $\frac{N}{50}$ iso-valeric acid, where penetration requires 1.5 minutes.

² Experiments with earthworms also disclose certain important deficiencies in the method whereby the animals to be tested are entirely immersed in the stimulating solution. If the reactions are to be studied quantitatively, there are features undoubtedly obscured by this procedure. For example, the time elapsing between the complete immersion of an earthworm (*Eisenia foetida*) in a $\frac{N}{10}$ lithium chloride solution and the instant the first writhing movement appeared, was found to be at least ten times greater than the reaction time of the same worm when part of it (the anterior end) was stimulated. The speed of reaction is in part conditioned by the number of receptors affected, but this does not analyze the situation completely. It is quite probable that the responses observed by Coghill are not at all due directly to sensory stimulation by acid.

³ I have observed that under certain circumstances *Ptychodera* sp.—a Bermudan enteropneust—reacts to chemical irritation by extruding a yellowish pigment. There is ground, however, for believing that this is an instance comparable to that of *Lumbriconereis* (Kschischkowsky, '12), in which K salts have apparently a more or less specific action in producing the response.

For $\frac{N}{10}$ HNO_3 the penetration time found (*Chromodoris* tissue) was 3.5 minutes; for $\frac{N}{20}$, 4.3 minutes; whereas *Necturus*, under water, was found to react to $\frac{N}{10}$ HNO_3 (0.5 cc. of the solution being applied with a pipette) in 1.5 seconds when stimulated on the dorsal surface of the head, 5 seconds on the lateral mid-body surface, and 2 seconds on the tail; with $\frac{N}{20}$ HNO_3 the reaction times for corresponding locations were 5, 10, and 6 seconds respectively.⁴ Even here, though the pipette tip was held within approximately a centimeter of the animal's surface, the concentration of the solution in the pipette does not represent the concentration which actually reaches the stimulated surface, as already noted by Parker ('12, p. 222). The conclusion must therefore be, that acids do not penetrate the skin with sufficient rapidity to affect the cells of the germinative layer. This conclusion must also be extended to alkalies, since Harvey ('10) and others have shown the high impermeability of cells to strong hydroxides.

IV. In the light of the evidence just discussed, it seems improbable that the high concentrations of irritants employed by Parker and others in studying reactions attributed to the common chemical sense produce any violently disruptive effects when applied to the skin of aquatic animals. Indeed, so far as concerns the skin as a whole, they do not penetrate at all,⁵ and the cells of the germinative layer cannot be held to be exposed to their action.

This conclusion was verified, in the case of the spinal frog, by experiments of the following type:

1. The reaction time for the withdrawal of the frog's foot from $\frac{N}{10}$ CuSO_4 , is about 7 seconds (at 24°9). After being withdrawn from the solution, the feet continue for some time to be spasmodically contracted. These subsequent contractions are entirely inhibited the instant a foot, just retracted from CuSO_4 ,

⁴ The reaction times were measured at 20°, while the penetration of the acid was studied at 27°; the speed of penetration decreases markedly with falling temperature.

⁵ The mode of action of the stimulating agent on the individual receptor is entirely another question.

is dipped into a weak solution of $K_4Fe(CN)_6$, which precipitates the copper held by the mucus of the foot. A similar result was obtained with copper acetate. Washing the foot with distilled water does not lead to a cessation of the contractions, because, after exposure of the foot to certain solutions (Loeb, '05), water stimulates.

2. After two to four stimulations of the frog's foot by relatively strong solutions of either copper acetate or ferric sulphate, the stimulated area was sectioned and tested microchemically for the penetration of the copper or iron. The metals were found in the mucus of the surface of the foot, and in several instances doubtful traces were discovered between cells of the extreme outer portion of the epidermis. No evidence was found of any disruption of the germinative layer.

3. As a stimulating agent whose penetration would readily be visible, the action of picric acid was studied. The outcome of experiments with this substance at several concentrations may be illustrated by the records here copied:

Experiments H and L. Picric acid, $\frac{M}{50}$. R. T. = Reaction time in seconds.

Successive tests at 5 min. intervals. N. R. = No reaction.

NO.	R.T.		NOTES
	H	L	
1.....	7.8	2.1	No staining.
2.....	12.8	6.6	Slight staining.
3.....	16.8	7.8	Staining progressively brighter.
4.....	30.2	11.8	
5.....	40.0*	24.6	
6.....	45.0	43.2*	
7.....	N.R.	N.R.	No reaction to $\frac{N}{5}$ formic acid.

* Not reactive to pinching beyond this point.

From these and similar tests, it was concluded that the penetration of the stimulant rapidly renders the frog's foot less re-

active by killing the superficial cells. In the case of pieric acid it is probably the H ion which is mainly concerned in stimulation, since $\frac{M}{50}$ ammonium pierate is entirely ineffective, neither does it easily stain the frog's foot. This does not, however, signify that other acids behave as does pieric, since as many as 20 successive reactions may be obtained from $\frac{N}{20}$ HCl . Moreover, the staining is not directly correlated with the stimulating effect, since the skin of the frog's foot was stained by immersion for 3 minutes in $\frac{M}{200}$ pieric acid without producing any reaction. The skin of *Necturus* and the catfish may be stimulated with $\frac{M}{50}$ pieric acid without producing any visible stain.

V. I have repeated on the frog's foot the experiment of Sheldon ('09) and Cole ('10) regarding the separation of tactile irritability and responsiveness to irritating solution, by treatment with cocaine. A 0.5 per cent solution of cocaine hydrochloride was used, and the tests were made by comparing at intervals the reactions of the cocainized foot with those of the untreated one. After about 20 minutes' immersion, the reaction time of the cocainized leg to $\frac{N}{20}$ formic acid (chosen as a powerful stimulant) was usually twice that of the normal foot; after about an hour, varying in some tests to an hour and a half, the cocainized foot no longer reacted to pinching, but gave good responses to the acid with reaction times of 10–15 seconds, still about twice the reaction time of the non-narcotized leg.

It is therefore possible, I believe, to effect experimentally a separation of sensitivity to mechanical and to chemical stimulation in the frog's foot.

There can be no question of the distinctness of the human sensation attributed to the common chemical sense (Parker, '12; Parker and Stabler, '13) as compared with any tactile sensation; and from tests made upon cocainized areas of my own mouth,⁶ I am certain that the two sets of receptors are not only qualitatively distinct as regards the sensations with which they are connected, but also may be separated by the use of cocaine.

⁶ These tests concerned mostly the inner surface of the cheek.

SUMMARY

1. There is positive evidence, in the case of alkalies, acids, and certain salts, that solutions supplying stimuli for the common chemical sense do not penetrate the skin of aquatic animals, nor when applied from a pipette do they damage the skin to any extent.

2. There is consequently no ground for Coghill's assumption that the cells of the germinative layer of the epithelium of fishes and amphibians are exposed to the action of the stimulating agent and thereby disrupted; and there is no histological evidence of disruption.

3. The reactions attributed to the common chemical sense depend upon a group of sense organs distinct from those sensitive to mechanical stimulation.

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STUDIES ON THE SPINAL CORD AND MEDULLA OF CYCLOSTOMES WITH SPECIAL REFERENCE TO THE FORMATION AND EXPANSION OF THE ROOF PLATE AND THE FLATTENING OF THE SPINAL CORD¹

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EIGHTY-SEVEN FIGURES

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INTRODUCTION

This paper has grown out of the study of the caudal heart and the spinal cord and nerves related to it in *Polistotrema*. Parts of that study which are not yet completed will be published later and will deal with the origin, distribution, and phylogeny of the spinal nerves; the origin of muscle sense organs in connection with the specialized muscles of the caudal heart; and

¹ A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy, Department of Anatomy, The University of Minnesota.

certain ganglion cells, possibly sympathetic, which are found along the course of the spinal and vagus nerves. The present paper includes a study of the mode of origin of the fourth ventricle in several groups, an account of a structure similar to the fourth ventricle found in the caudal part of the spinal cord of an adult *Polistotrema*, and a study of the development of the central canal, and the causes underlying the flattening and the ventral indenting of the spinal cord in Cyclostomes.

Material and its preparation

Since the material for this paper came from such diverse sources, and such different modes of technique were employed, too much time and space would be consumed if a detailed description were given of each method employed. With the exception of the *Petromyzon* material, which was fixed in Flemming, corrosive-acetic, and Perenyi's fluid, my own material was fixed either in Tellyesnický's or Bouin's fluid. It was sectioned after paraffin or the combined celloidin-paraffin method of imbedding. The latter method gave by far the better results, since it appears to have all of the advantages of celloidin in causing almost no shrinkage and its ability to hold yolk granules and blood corpuscles intact; besides allowing the sections to be cut as thin as paraffin alone, and causing no more difficulty in manipulation. For the most part the sections were stained in Heidenhain's iron hæmatoxylin and counterstained in an alcoholic solution of orange G plus a little acid fuchsin. In a few instances, in very young *Petromyzon* embryos, where all the tissues were filled with yolk, carmine and hæmalum were used to advantage.

Acknowledgments are due to Prof. T. G. Lee for the gift of a very complete series of *Petromyzon* embryos, which were obtained from the Connecticut River. Also to Prof. R. E. Scammon for the loan of his very complete serial collection of *Squalus* embryos, and to Prof. J. B. Johnston for the use of a similar serial collection of *Amblystoma* embryos. The splendid series of human and pig embryos belonging to the Institute of

Anatomy of which Prof. C. M. Jackson is director, were especially valuable.

It is a great pleasure to the writer to have this opportunity of expressing his obligations to Prof. J. B. Johnston for his valuable suggestions and friendly criticism of this work.

A model of the caudal end of the spinal cord from a region a little in front of the caudal hearts to its extreme tip was prepared in four sections from the 20 cm. *Polistotrema* series. Also a model in two pieces was prepared of the cavity of the so-called first roof plate expansion and the enlarged central canal of the above mentioned series of *Polistotrema*. These models were constructed out of blotting paper after a modification of the Born method. Tracings of each section were made on ordinary writing paper with the aid of an Edinger-Leitz drawing apparatus, using a magnification of 100 diameters, and afterward each tracing was carefully checked up for accuracy with a higher magnification. At the very outset two base or projection lines were drawn on the first tracing, one following the median longitudinal plane, and the other a horizontal line drawn at right angles to the first line, passing along the ventral border of the notochord. These projection lines were added by pencil to all of the succeeding tracings after the following manner: The second tracing was carefully fitted over the first, after placing both over a plate glass covered box, containing an electric light reflected upward, and in like manner these lines were added to the third tracing from the second, and so on to the end of the series. Then with the aid of carbon paper these tracings and projection lines were transferred to sheets of blotting paper, having a definite thickness, previously determined after the following method. When thoroughly cooled after an immersion in melted paraffin, the blotting paper selected should have a thickness, equal to the thickness of the section multiplied by the magnification used, which in this case totalled 1.5 mm. The sheets of blotting paper containing the transposed tracings were then immersed in melted paraffin, drained and cooled. After which the outlines of the tracings were cut out with a sharp scalpel, and the sections were built up in regular order. In

part to maintain this regular order and in part to add strength to the model, a copper wire was inserted through each section, passing through the point of intersection of the two projection lines. After a certain number of sections had been strung on this wire they were securely fastened to each other by pins.

HEREDITARY AND MECHANICAL CAUSES UNDERLYING THE
FORMATION OF THE FOURTH VENTRICLE AND THE
TELA CHORIOIDEA

To the writer this problem appears to be primarily phylogenetic rather than ontogenetic; consequently this study begins with the lowest vertebrates, and is approached as a problem of evolution.

1. Roof plate of the medulla oblongata

A. Amphioxus. Unfortunately I have not had access to any embryological *Amphioxus* material so that my inferences will have to be drawn entirely from adult material.² At the outset it can be maintained with a considerable degree of safety that the adult *Amphioxus* brain contains nothing which can be homologized with the fourth ventricle of higher vertebrates. Figure 67, which passes through the highest point of the anterior ventricle (V_1) demonstrates clearly that there is nothing here comparable with the fourth ventricle. For the dorsal portion of the cavity is much narrower than the ventral portion, and there is absolutely nothing in the way of a thin and expanded roof plate. In fact, the dorsal portion of the cavity is fairly filled up with processes from ependymal cells.

A few sections behind the first ventricle there appears a small isolated cavity in the roof plate region of this series, designated as the posterior ventricle (fig. 68, V_2), and a considerable distance behind this cavity, there is a second dorsal cavity, also designated as the posterior ventricle (fig. 69, V_2). This is somewhat larger than the previous cavity, and can be readily located

² In order to eliminate minor details of description from the text, very complete and detailed descriptions of the figures have been given at the end of the paper, to which the reader's attention is directed.

from its position immediately above a dorsal group of large nerve cells (*M.C.*). From the figures by Hatschek, Willey, Sterzi, and others it is evident that these isolated roof cavities described above, were once a part of a common central cavity of the embryonic brain, which later in development, became isolated through an invasion of ependyma, and it is entirely possible that this region of the central cavity in the embryo was much more suggestive of the fourth ventricle. Judging from the adult alone they may be looked upon merely as vestiges of the embryonic central canal.

B. Polistotrema (Bdellostoma) My embryonic material of *Polistotrema* confirms the statements of Price, von Kupffer, and Dean that *Polistotrema* possesses well-developed ventricles in the embryo; the expansion being fully as great as in a similar stage of *Petromyzon*. As development proceeds the lateral plates increase in thickness from additions of fibers and cells until the fourth ventricle becomes reduced to a canal, but little larger than the central canal of the spinal cord.

Sanders, Holm, Miss Worthington, Sterzi, Cole, and Nicholls describe and figure the fourth ventricle about as it is shown in figure 63. There is some little discrepancy in the terminology used, due largely to the differences of opinion as to whether or not *Polistotrema* has a cerebellum. If the posterior lobes of the mesencephalon should in the light of future investigation turn out to be a cerebellum, then the boundaries of the metencephalon will have to be carried further forward than we have indicated, and the so-called sinus mesocœlicus of Nicholls (*S.M.*) will have to be called the anterior dilation of the fourth ventricle of Miss Worthington's description.

My transverse series through the brain of *Polistotrema* show the condition of the ventricles to be almost identically the same as Sterzi and Nicholls found them. Until the embryological and functional areas of the brain have been better worked out it seems advisable to the writer to let the posterior border of Nicholls' sinus mesocœlicus (fig. 63, *S.M.*) mark the boundary line between the mesencephalon and the metencephalon, and to regard his isthmie and ventricular canals (figs. 63 and 65, *A.V.*)

as dorsal and ventral portions of the anterior end of the fourth ventricle. For the reason that in *Polistotrema* they extend some distance behind the posterior tip of the mesencephalon, and the dorsal or posterior canal is shown in transverse section (fig. 65) to lie close to the dorsal surface, which is then the only part of the fourth ventricle to retain the characteristic dorsal position of the higher vertebrates. These two canals appear in this series about as Nicholls has described them, the dorsal (isthmie) is the larger and contains Reissner's fiber. Although probably subject to a considerable variation, these canals apparently extend further caudad in this series than Nicholls represents them. Also in this series the dorsal canal (isthmie) gives off one or two branches at the level of the posterior tip of the mesencephalon (cerebellum of Miss Worthington), which run parallel to the main canal, but a little to one side and below (fig. 65, *A.₄V.*). After travelling side by side for some little distance in a mass of spongy ependymal tissue close to the roof plate, they reunite in the dorsal canal, and soon afterward both the dorsal and ventral (isthmie and ventricular) canals unite in a common canal, which is little if any larger than the central canal of the spinal cord. This constricted portion of the fourth ventricle (fig. 63) continues caudad, rather deep-seated in a mass of loose vascular ependyma, until the posterior end of the medulla is reached, where it expands into a much larger vesicle or sinus, designated as the posterior dilation of the fourth ventricle (figs. 63 and 66, *P.₄V.*). Behind this it soon tapers down into the ordinary central canal of the spinal cord.

A glance at figures 63 to 66 suffices to show that the fourth ventricle of *Polistotrema* is greatly reduced as compared with that of *Petromyzon*. This is due probably to the rapid increase of fibers and cells in the lateral plates. Notwithstanding this reduction in size and general alteration in appearance and structure, the walls of the fourth ventricle in *Polistotrema*, although representing a greatly modified chorioid plexus, are unquestionably capable of producing cerebro-spinal fluid. The posterior dilation of the fourth ventricle (fig. 66, *P.₄V.*) contains cerebro-spinal fluid (*S.C.F.*) in the form of a deeply staining feltwork,

which is not ependymal cilia or a tangled Reissner's fiber. Also throughout its entire length, as was noted by Sterzi, the fourth ventricle is enveloped by a rather thick layer of spongy and very vascular ependyma, which would be distinctly favorable for infiltration and possibly for secretion into the ventricle. This rich blood supply is from the blood vessels and sinuses traversing the meningeal membranes, and especially from two large arteries (A. rhombencephalica of Sterzi), one of which appears in figure 64 (M.A.). Also it would be quite possible in the anterior part of the fourth (A.₄V.) for the various canals, which run close to the dorsal, to receive infiltration direct from the outer meningeal blood and lymph sinuses.

Coagulated cerebro-spinal fluid is also to be seen in reduced amounts in the mesencephalic ventricles designated as the posterior mesocœle and the sinus mesocœlicus. They are also surrounded by a layer of vascular ependyma, which, while much thinner than the corresponding layer of the fourth ventricle, doubtless functions as a cerebro-spinal fluid forming organ.

A careful examination of this peculiar modification of the chorioid plexus of the fourth ventricle in *Polistotrema* leads one to believe that this is not as efficient an organ for the production of cerebro-spinal fluid as the more expanded tela chorioida of *Petromyzon* and higher vertebrates.

C. Petromyzon. *Petromyzon* is apparently the best type that could be selected for obtaining definite information concerning the early history of the formation of the fourth ventricle and its expanded roof plate. 1) It is the lowest living vertebrate that possesses a well-developed fourth ventricle and expanded tela chorioida in the adult. 2) At no times does the medulla have a pontine flexure. 3) Its central nervous system remains a solid cord of ectoderm until after the cranial and spinal ganglia are well-differentiated. For these and other reasons the early history of the fourth ventricle in *Petromyzon* has been studied in the effort to determine the essential factors involved in its appearance and growth.

In order to arrive at the fundamental factors involved in the anlage and development of the fourth ventricle in *Petromyzon*

it is necessary to go back in the ontogeny of the central nervous system to the time when it was a solid cord. Of my embryos which represent stages killed at 5, 7, 9, 10, 11, 12, 14, 16, 18, 20, and 26 days after fertilization, all of the 5 day series revealed the central nervous system as a solid cord or keel of ectoderm cells, formed according to Calberla by a process of delamination. In places neural crest cells can apparently be seen budding off from the dorso-lateral surface of the brain and spinal cord almost identically as described and figured by von Kupffer ('90). It is obvious that the preponderance of yolk granules in all tissues makes accurate observation in these stages very difficult. The majority of the 7 day embryos disclosed a central canal either formed or in the process of formation. Out of a great number of series of 10 day embryos, three were found in which the opening of the central canal had been considerably retarded. In one series the central nervous system was still a solid cord of cells, and in the other two the central canal was in the early stages of formation. These series were selected in preference to similar stages of 7 day embryos for the reason that less yolk was present to obscure the various structures.

To facilitate comparison of similar sections of successive stages, figures 33 to 38 and figures 40 to 53 have been so arranged as to bring several regions of series in a horizontal row, while successive stages of the same region are placed in a vertical row. The conditions at the level of the trigeminal, auditory and vagal ganglia are thus readily compared.

In figure 32 we have a section through the medulla in the region of the VIII ganglion, which portrays very well an extremely early stage in the formation of the central canal. Here the medulla will be seen to be composed of a mass of nuclei imbedded in a syncytium of protoplasm. Excepting in the roof and floor plates, the nuclei have migrated some little distance to either side of the median sagittal plane, presenting the appearance of a rather broad light line surrounded by nuclei. The center of this light streak of protoplasm discloses a very conspicuous furrow or seam (*C.C.S.*), which appears in every section of the central nervous system of this series, very much as it looks in

this section. This furrow or seam represents the position of the future central canal. The protoplasm adjacent to this seam is sufficiently granular to suggest a secretory function at this early stage.

Figures 33 to 35 also taken from a 10 day *Petromyzon* series exhibit a slightly later stage in the formation of the central canal. Conspicuous cavities (C.C.) have appeared in the dorsal and ventral portions of this furrow, which are not only visible throughout the entire medulla, but are continuous throughout the spinal cord. It should be recorded that a few sections through the medulla possessed slightly larger dorsal and ventral cavities than were represented by these figures, but in no case had they approached each other close enough to unite. In other respects, excepting possibly for a few more fibers in the marginal layer, the structure of the medulla has remained about the same as in the previous series. Emphasis should be made of the fact that each of these cavities, when examined with a higher magnification, reveals a certain amount of fine granules, which in some cases may have completely filled the cavity, while in others they are confined to the outer edge, leaving a clear space in the center. The presence of these granules here suggests two processes: 1) disintegration of the central protoplasm, and 2) products of secretion. The lateral migration of the nuclei could be utilized to support either inference. That such a migration of nuclei would be favorable for disintegration is self evident, and secretory cells are usually characterized by having their nuclei somewhat remote from their lumina.

In the 11 day series (figs. 36 to 38) we find that the two isolated dorsal and ventral cavities of an earlier stage have not only united and formed a cleft-like cavity, which may now be designated as the typical embryonic central canal, but that the original dorsal and ventral cavities of this canal, especially the dorsal, have increased notably in size. The narrow central portion (figs. 37 and 38) indicates that this is the place where the protoplasm was last to separate. It should not be confused with a similar condition that occurs later, when the central and ventral portions of the lateral plates migrate inward, fuse, and

completely obliterate that portion of the embryonic central canal. Aside from the change in the central canal the size of the medulla has increased in all directions, but especially laterally, due doubtless to an increase in the number of nerve fibers and cells in the lateral plates. Both roof and floor plates are very thin, comprised of about one layer of nuclei each. The ventral plate may be slightly thicker, due to the addition of a few nerve fibers to the outer layer. Absolutely no stretching of the roof plate has occurred, indicating that it is not under any marked internal pressure. In figure 37 (*B.V.*) branches of the inter-segmental blood vessels have stretched out toward the roof of the brain, but at this stage they are too remote from the roof plate to be very active in infiltration. Figure 39 makes clear that the spinal cord has made equal progress with the medulla in developing a cleft-like or typical embryonic central canal. In the cord the dorsal and ventral enlargements of the central canal are shown to be of about equal size.

An additional day (figs. 40 and 41) discloses considerable expansion of the central canal throughout, with the exception of the extreme ventral portion. Very noticeable is the increase in size of the dorsal portion, the future fourth ventricle (*cavità della tela corioidea* of Sterzi). The roof plate exhibits no signs of stretching. Up to this stage the expansion appears to be due to the migration of the ependymal cells upward and outward, rather than to pressure within. The floor plate will be seen to have increased considerably in thickness through an addition of fibers to its marginal layer, which would obviously tend to make the floor plate more resistant than the roof plate to pressure from within from this time on. Both lateral plates disclose a remarkable growth in thickness due to an addition of both fibers and cells, some of which, however, must be attributed to the fact that the head was sectioned somewhat obliquely (note in fig. 40 that the V and VIII ganglia appear in the same section). But little progress has occurred in the development of the inter-segmental blood vessels, so that Sterzi's conjecture, that the embryonic cerebro-spinal fluid does not differ in any way from the ordinary intercellular fluids, would probably hold, if it could be

established that the central protoplasm in disintegration contributed nothing to it. The spinal cord was found to be in about the same condition as the 11 day series.

Moreover an 18 day series (figs. 42 to 44), some 6 days older than the last stage compared, shows practically no change in the shape and size of the central canal or expansion of the roof plate, despite the fact that a notable increase of fibers had occurred in the ventral and median portions of the lateral plates. It should also be recorded that the intermediate stages, as represented by the 14 and 16 day series, exhibited a like state of the central canal. A further interesting condition is revealed from a section of the spinal cord of this series (fig. 45). Here the lateral plates have expanded to such an extent from the formation of fibers in the marginal layer, that the inner surfaces of their central areas have nearly formed a complete concrescence at the center of the original central canal. In other words, the inner surfaces of the lateral plates have met, and are about to fuse, leaving dorsal and ventral cavities (*C.C.*). The ventral one of these cavities will persist as a permanent central canal. In *Petromyzon* this partial closure of the original embryonic central canal of the spinal cord may have considerable bearing on the increase of internal pressure of cerebro-spinal fluid on the walls of the medulla, during this and later stages. We may regard the period from about the 12th to about the 18th day as a period of rest in the formation of the fourth ventricle in *Petromyzon*. During this time the processes which would tend to expand the central canal have been met with equal counter forces, which would make for closing it up.

Transverse sections through the medulla of a 20 day *Petromyzon* series (figs. 46 to 48) demonstrate a marked change in the medulla and its central cavity, which cavity has now assumed the form of a fourth ventricle. A marked increase has taken place in the number of fibers in the ventral plate and in the ventral portion of the lateral plates. This together with pressure from the growing auditory vesicles (*Aud.V.*) and the notochord, has produced a nearly complete concrescence of the corresponding middle and ventral portions of the inner surfaces

of the lateral plates. Had a similar increase of nerve fibers occurred in the dorsal plate and in the dorsal portion of the lateral plates, the central canal of the medulla might have been completely obliterated. On the contrary, the dorsal cavity and the roof plate have been slightly expanded, apparently through an increased internal pressure from the embryonic cerebro-spinal fluid. One evidence that increased pressure may exist was found in the marked decrease in the size of the central canal of the cord and of the ventral part of the canal in the medulla region.

A second evidence is found in the changes that the walls of the fourth ventricle are undergoing preparatory to becoming a functional organ for the production of cerebro-spinal fluid. In connection with the expansion of the roof plate it will be seen that the dorsal mesenchyme has become decidedly vascular (fig. 46, *B.V.*), making easy an infiltration process into the ventricle. Also ependymal cells, surrounding the ventricle, are taking on form and are probably assuming a secretory function, if they have not acquired one previously. From the recent work of Dandy and Blickfan based on the action of certain drugs and on the chemistry of cerebro-spinal fluid, it is evidence that cerebro-spinal fluid must be a product of secretion as well as of infiltration and diffusion. It is a well-established physiological fact that certain secretory cells, as for example the salivary glands, may assume a definite polarity, and produce a secretion against a very strong pressure, even stronger than that of the blood. Some investigators hold that lumina in glands are the result of pressure from secretion.

In the light of these facts, it is fair to assume that the marked lateral expansion of the fourth ventricle and its roof plate exhibited in the 26 day series (figs. 50 to 52) are the direct result of internal pressure caused by the marked increase in cerebro-spinal fluid. The general increase in size of the ventricle together with the marked convexity of the roof plate and the concavity of the internal surface of the lateral plates certainly suggest internal pressure from the cerebro-spinal fluid. It should be noted in figures 50 to 52 that this expansion of the

fourth ventricle has produced a considerable secondary splitting in the concrescence of the lateral plates recorded for the previous series. Apparently this fissure did not penetrate so deeply in the region of the auditory vesicles (fig. 51, *Aud.V.*) as it did in front of them (fig. 50) or behind them (fig. 52), which probably indicates that the growth of the auditory vesicles in some way operated against the further splitting of medulla. It is significant that the expansion of the roof plate and the development of its mechanisms for infiltration and secretion occur at a time prior to the entrance of blood vessels into the wall of the brain and cord. The large production of cerebro-spinal fluid at this time is evidence that it serves some nutritive function.

In figure 54 we have a median sagittal section through the brain of a 26 day *Petromyzon*, representing a stage similar to that of figures 50 to 53. Especial attention is called to the fact that *figure 54 demonstrates conclusively that the pronounced roof expansion displayed in figures 50 to 52 has occurred without the aid of a pontine flexure.* The slight convexity of the floor of the medulla can be attributed to the increase of fibers. Also earlier and later series revealed that *Petromyzon* possesses no pontine flexure. Attention should be called to the fact that the marked convexity of the cephalic end of the thin roof plate of the fourth ventricle (*C.C.*) in figure 54 gives every appearance of being under internal pressure from cerebro-spinal fluid. This section also makes clear, as Sterzi has previously shown, that the fourth ventricle is formed from the dorsal portion of the embryonic central canal; while the central canal of the cord is formed from the ventral portion.

The following observations were noted in connection with the appearance of the fourth ventricle in *Petromyzon*, beginning at a stage when the medulla was a solid cord of undifferentiated nuclei in a synectium of protoplasm: 1) The ependymal nuclei migrated a short distance to either side of the median sagittal plane leaving a narrow strip of granular protoplasm in the center. 2) A median sagittal furrow or seam appeared in the central protoplasm, extending from the roof plate to the floor plate. 3) An isolated cavity appeared at the dorsal and ventral ends

of this furrow. 4) The two cavities became connected, forming a cleft-like canal, designated as the typical embryonic central canal. This canal seems to be formed by a disintegration of the central ends of the ependymal cells, now in the form of a syncytium. 5) A considerable increase in the size of the dorsal portion of the central canal occurred through the upward and outward migration of some of the roof plate nuclei and a disintegration of the inner protoplasm. No similar expansion of the ventral portion of the central canal of the spinal cord took place because its marginal layer became reinforced very early by the addition of nerve fibers. 6) Following the formation of the central canal of the medulla there was an increase of cells and fibers in the lateral walls, but for the space of about six days there was little change in the size of the central canal. 7) Next a very pronounced increase of fibers occurred in the median and ventral portions of the lateral plates, which brought about a complete concrescence of the corresponding inner margins of the lateral plates. 8) A sufficient amount of cerebro-spinal fluid was formed by infiltration and secretion to produce a marked expansion of the fourth ventricle. This expansion pushed apart the dorsal portion of the lateral plates, which had not been thickened by an addition of fibers, and stretched the roof plate to a much greater width. 9) Along with this expansion a secondary splitting of the concrescence noted in (7) took place. This fissure did not penetrate so deeply in the region of the auditory vesicles on account of the mechanical obstacle offered by these vesicles. 10) This expanded roof plate apparently assumed the function of producing cerebro-spinal fluid at a time previous to the entrance of blood vessels into the central nervous system, when its nutritive function would be of importance.

From a review of the main points in the development of the fourth ventricle in *Petromyzon* we are warranted in concluding: 1) That a well-developed fourth ventricle and tela chorioidea were formed in one of the lowest living vertebrates, *Petromyzon*, without the aid of a pontine flexure. 2) The best suggestion that has been given for the appearance of such an organ in the medulla rather than elsewhere in the central nervous system is

that the roof plate has been weakened more in the medulla region through a greater migration of neural crest cells. This explanation, however, is not entirely satisfactory; since it was shown for *Petromyzon* (fig. 32) that the cranial ganglia were well-differentiated while the medulla was still a solid cord of cells. If then this were the determining factor it must have cast its shadow a long way ahead. Also it is apparent when the difference in size is considered between the medulla and the spinal cord that there are relatively no more neural crest cells extruded from the medulla. 3) The roof expansion appeared at the same time in the region of the X ganglion as in the region of the VIII and V, and developed at a uniform rate throughout the medulla. 4) Two factors are evident in the formation of the fourth ventricle: first, an upward and outward migration of the roof plate nuclei followed by a disintegration of the inner protoplasm; second, internal pressure exerted by the rapidly increasing cerebro-spinal fluid, infiltrated and secreted by the roof plate itself.

D. Selachians and Amphibia. As types of these classes I had access to very complete sets of serial sections of *Squalus* and *Amblystoma* embryos, and to one transverse series of a 15-mm. *Necturus*. A careful examination of this material contributed nothing new to the ontogeny of the fourth ventricle. It was possible, however, to confirm in these forms many of the conclusions arrived at in *Petromyzon*. In both *Squalus* and *Amblystoma* the much earlier appearance of the central canal brings about a much earlier and more extensive expansion of the fourth ventricle. The sections shown in figures 72 and 73, while possessing enormous expansions of the roof plate, exhibit less progress in the differentiation of the structure of the medulla than was revealed in a 12 day *Petromyzon* (fig. 41), in which very little or no expansion had taken place in the roof plate.

In *Squalus* and *Amblystoma* the typical embryonic central canal, in the form of a vertical cleft, is a comparatively late production. The canal appears soon after the neural folds coalesce as a horizontal cleft, its presence often being indicated only by a layer of pigment. This cleft becomes elliptical, then more or less circular, and finally changes to a vertical or dorso-

ventral cleft. These changes in the shape of the central canal suggest a migration of cells, rather than a disintegration process or a splitting apart of the walls as a result of pressure from the secretion of cerebro-spinal fluid. The earliest *Squalus* series showed an elliptical canal with its longest axis horizontal, this changed to circular, and finally to the so-called typical embryonic central canal (dorso-ventral cleft canal). For a time in the medulla of *Amblystoma* the roof plate is much thicker than the floor plate, a condition which can be attributed to the presence of a number of neural crest cells in the roof plate, easily distinguishable from the other cells by their large size and spherical form. After the neural crest cells had been entirely separated from the roof, the roof and floor plates are found to be about equally thick. The same thinning out of the roof plate occurs in *Squalus* in part through the giving off of neural crest cells, which in this species are indistinguishable from the other cells of the medulla. It is evident in *Amblystoma* (figs. 74 and 83, *R.P.*) and also in *Squalus* that the roof plate of the medulla has become no thinner than the roof plate of the spinal cord through the throwing off of the neural crest cells. There is no evidence that the roof plate in the medulla is rendered any weaker or any more susceptible to expansion than is that of the spinal cord, through the migration of ganglion cells.

Apparently the first expansion of the roof plate in *Amblystoma* (fig. 74, *R.P.*) and in *Squalus* is produced as in *Petromyzon* by an outward and upward migration of the roof plate cells. The later, more pronounced expansion and stretching of the roof plate can also be ascribed, as in *Petromyzon*, to internal pressure due to a decided increase of cerebro-spinal fluid (see fig. 72 for *Squalus* and 73 for *Neeturus*). In both species the dorsal and middle portions of the embryonic central canal of the spinal cord are obliterated by an inward growth of the lateral plates through an addition of fibers. Pressure on the cerebro-spinal fluid might be increased from that source. Also a similar effect would be produced through a marked proliferation of fibers in the medial and ventral portions of the lateral plates of the medulla, which brings about a coalescence of the ventral por-

tions of the lateral plates in *Squalus* (fig. 72, C.C.C.) similar to the condition in *Petromyzon*. In *Necturus* (fig. 73) and in *Amblystoma* the roof plates are expanded to such an extent that no fusion of the lateral plates takes place; nevertheless, the thickening of the walls tends to bring about a reduction in the size of the fourth ventricle and consequent pressure on the cerebro-spinal fluid. The probability of a decided increase of internal pressure from the cerebro-spinal fluid resulting from the reduction in caliber of the embryonic central canal of the spinal cord by at least two-thirds, is more evident, if attention is directed to the fact that the length of the central canal of the spinal cord is fully twenty times that of the fourth ventricle. In both forms the above changes took place before the blood vessels had reached the dorsal surface of the roof plate or entered the medulla. A median sagittal section through a *Squalus* embryo of the same stage as figure 72 shows that no pontine flexure has appeared.

E. Pig embryos. For making observations on the development of the tela chorioidea of the fourth ventricle in mammals the writer had access to a very complete set of frontal sections of pig embryos from a stage of 4 or 5 mm. up to 14 mm. embryos.

The earliest section (fig. 75), which is from a 4 or 5 mm. pig, discloses that the fourth ventricle has only begun to expand. A large part of this expansion could be attributed to an outward and upward migration of the roof plate cells, and a part to internal pressure from cerebro-spinal fluid, of which traces are beginning to appear as a coagulum (S.C.F.). It should be noted that the first blood vessels are appearing above the basal portions of the roof plate, while none have at this stage entered the medulla. Also the ependymal cytoplasm is sufficiently granular to suggest a secretory process, and finally the roof plate has begun to expand before any nerve fibers have appeared in the marginal layer.

A transverse section of the medulla of a 6 mm. pig (fig. 76) revealed a considerable expansion of the roof plate without the aid of a pontine flexure. It is apparent that an increase in the amount of cerebro-spinal fluid is the main factor in bringing

about this pronounced expansion of the roof plate. Since the closure of the dorsal portion of the embryonic central canal of the spinal cord occurs much later in embryonic life, no increase in pressure from cerebro-spinal fluid could take place from that source. Also no increase was shown in the number of blood vessels outside the roof plate, and no blood vessels had entered the medulla. Consequently the only means of an increase of cerebro-spinal fluid would be through secretion and a slight infiltration from the blood vessels. This section demonstrates very strikingly, even more so than figure 75, that the roof expansion begins very early in the development of the pig's medulla, as is evident from the fact that nerve fibers have only begun to appear in the marginal layer.

Between the 6 mm. and 7 mm. stages there occurs a marked increase in the blood vessels in the mesenchyme above the roof plate and a few blood vessels are entering the substance of the medulla. There can be no question that the roof plate is now an efficient organ for the production of cerebro-spinal fluid, and there is a noteworthy increase in the amount of coagulum in the cavity (figs. 75, 76, and 77). The first embryonic cerebro-spinal fluid, probably a mere intercellular fluid, showed little or no coagulum, from the method of fixation and staining used. This may indicate a relative increase of the elements formed by secretion. Figure 77 shows that a marked expansion of the roof plate has occurred in the 7 mm. pig. Since the 7.5 and 8 mm. pig embryos have very small pontine flexures, it is evident that this pig embryo has developed a well-expanded roof plate and choroid plexus without the influence of a pontine flexure.

The roof plate in the 10 mm. pig (fig. 78, *R.Ex.*) and in the 14 mm. pig (figs. 79 and 80, *R.Ex.*) has undergone a decided expansion, especially in a dorsal direction. This final expansion of the roof plate is unquestionably due to the action of the pontine flexure upon a fourth ventricle filled with cerebro-spinal fluid already under moderate pressure. It would have been impossible for a pontine flexure acting alone on an empty fourth ventricle, as would be implied from His' experiments with bending an empty rubber tube slit dorsally, to have brought about

the dorsal expansion of the roof plate exhibited in figures 79 and 80. Apparently this expansion of the roof plate in the pig has been gradual, for absolutely no stretching of the roof plate has occurred, except in one place, namely in its central anterior portion (fig. 79).

A study of these sections has disclosed a direct relationship between the expansion of the roof plate and the amount of visible coagulum in the ventricle. Since coagulum does not appear in sections of the early fourth ventricle, but does appear after the tela chorioidea has attained the function of producing cerebro-spinal fluid (as is indicated by its vascularity and the granular appearance of the cells) it is fair to assume that the non-coagulable cerebro-spinal fluid found in sections of the early embryos is an embryonic cerebro-spinal fluid, which differs in no way from the ordinary intercellular fluid of other tissues. On the other hand, the coagulum seen in sections after the roof plate has reached the stage of a functional chorioid plexus is evidence of a chemical change in the fluid, which, if a product of secretion, is capable of exerting considerable internal pressure and consequent expansion of the roof plate.

It is apparent that the greater expansion of the roof plate in the pig is produced by the same factors as were recorded for *Petromyzon*, namely, an early migration outward of the roof plate cells followed by an expansion from within due to the formation of cerebro-spinal fluid, plus the action of a conspicuous pontine flexure on a fourth ventricle filled with cerebro-spinal fluid already under moderate pressure.

F. Human embryos. For this study an 8 and a 15 mm. transverse series and a 23 mm. frontal series were available. These embryos were too far advanced to show the earliest stages of the roof expansion of the fourth ventricle. If, however, the extreme posterior end of the roof plate of the fourth ventricle is examined in the 23 mm. embryo (figs. 26 and 27, *R.Ex.*), in the 15 mm. embryo (figs. 28 and 29, *R.Ex.*), and in the 8 mm. embryo (fig. 31, *R.Ex.*), which represents a region of the medulla little affected by the pontine flexure, it is apparent that the roof expansion was caused by identically the same factors as was

recorded for the pig embryos. In view of the facts, this explanation of the formation of the roof expansion seems more tenable to the writer than to attribute all, as His has done, to the action of a pontine flexure. For beyond question, a considerable expansion of the roof plate in the human medulla takes place before the pontine flexure appears.

2. *Description of three roof plate expansions of the spinal cord in the 20 cm. Polistotrema series*

An interesting variation (abnormality) was found in the spinal cord of a single specimen of *Polistotrema* (*Bdellostoma*), otherwise unusual. Certain structures appeared in the roof of the cord that were very similar to the tela chorioidea of the fourth ventricle, and will be described because of the light that they may throw on the origin of the tela chorioidea of the fourth ventricle.

What has been designated as the first roof plate expansion of the 20 cm. *Polistotrema* series is shown in the photographs of models 1 and 2 (figs. 1 and 2, 4 and 5, *R.Ex.*) to be an immense outcropping of the roof plate ependyma. Most unfortunately the anterior portion of this specimen, from which the series through the tail was taken, has been lost, so that it is impossible to state how much further cephalad this expansion of the roof plate extended, or whether there were other outcroppings of the roof plate in front of it as there are behind. It appears in the first model (fig. 2, *R.Ex.*) and in the first transverse section (fig. 10, *R.P.Ex.*) as a median mass, covering about one-half of the dorsal surface of the spinal cord; it then shifts gradually over to the right side (figs. 2 and 11); then gradually attains a median position. In this position it continues as far as the middle of model 2 (fig. 5), covering a large part of the dorsal surface of the spinal cord.

For the most part the roof plate expansion contains a cavity of considerable size, which is shown anteriorly in the cast (fig. 3, *C.C.Ex.*) and in transverse section (figs. 10 to 12) to be in direct communication below with the central canal. Posteriorly

such a connection is wanting (fig. 13). The fact that in various places (fig. 13) there is more or less of a string of ependymal cells between the walls of the central canal and the roof plate expansion suggests that in a more embryonic state an open communication existed between the roof plate cavity and the central canal, which became closed in the region of the posterior end of the roof expansion, and persisted in the anterior end. Another variation to be noted in the cavity of the posterior end of the roof plate expansion is that it contains numerous islands and promontories of ependymal cells and connective tissue, shown as white spaces in figure 6. These probably represent portions of the roof plate that have not been completely excavated to form a continuous cavity.

Transverse sections 11 to 13 show the cavity of this first roof plate expansion to be larger than the fourth ventricle in *Polistotrema* (figs. 64 to 66), and the whole structure more nearly resembles a typical fourth ventricle than does the fourth ventricle itself in this animal. For the most part the walls of the roof plate consist of true ependymal cells, differing in no way from those surrounding the central canal, except for their shorter peripheral processes. Posteriorly connective tissue takes the place of many of these cells. One of these cells is shown in figure 13A to be sufficiently granular to suggest a secretory function. Everywhere the walls of both the roof plate and the central canal are very vascular, suggesting a modified choroid plexus. Figure 13A will demonstrate the ease with which infiltration and diffusion could take place between the blood vessels of the roof plate and its cavity. In figure 11 a fold of the roof expansion, containing a blood vessel, will be seen extending into the cavity, and about it there is collected a mass of coagulated cerebro-spinal fluid (*S.C.F.*).

Of still greater interest are the two posterior outcroppings of the roof plates, designated as roof plate expansions 2 and 3 (figs. 4 and 5, *R.Ex. 2 and 3*). Since these two roof plate expansions are considerably smaller than the first, they can be compared directly with the roof plate of the rhombencephalon of any embryo.

The so-called second roof plate expansion appears in model 2 (figs. 4 and 5, *R.Ex. 2*) immediately behind the first. This is a much smaller outcropping of the roof plate. It contains a cavity (figs. 16 and 17, *C.C.Ex.*), which spreads out a little laterally and caudally, and communicates below with the central canal. This cavity is filled with a fine fibrillar feltwork that stains deeply with orange G, and which for the most part is coagulated cerebro-spinal fluid. The ependymal walls of both the roof expansion and the central canal are sufficiently vascular to suggest that we have here as in the previous roof expansion, a modified chorioid plexus which is producing cerebro-spinal fluid.

In model 2 (figs. 4 and 5, *R.Ex. 3*) the third outcropping of the roof plate is some little distance behind the second, about equaling it in size. This roof expansion has not been figured in transverse section, but from an examination of a graphic reconstruction of the central canal, three small isolated cavities were seen extending in a cephalo-caudal direction. The middle cavity was found to be in communication below with the central canal. It held cerebro-spinal fluid, and its walls apparently functioned in the production of the same.

It is evident that these three expansions of the roof plate are independent of one another. The arrangement of the three small isolated cavities in the third expansion seems to be an embryonic condition, and suggests that the larger cavities may have been produced by the union of several smaller roof expansions. It may be supposed that these expansions were formed by the multiplication of roof plate cells, which were pushed up in solid masses, in which vacuolization and confluence of adjacent cavities produced the larger cavities seen in the adult.

It is of interest to note that in this individual a posterior sinus (fig. 20, *S.T.*), probably representing the sinus terminalis of a normal individual, was isolated by the complete occlusion of the central canal by ependymal tissue. The ependyma surrounding this cavity is very vascular, and the cavity is distended with cerebro-spinal fluid. This sinus is much larger than the fourth ventricle in a normal individual.

When the roof expansions in the spinal cord of this series of *Polistotrema* are compared with the fourth ventricle of the higher vertebrate embryos, it is evident that the similarity is only superficial, for the later stages in the medulla oblongata (action of pontine flexure on a thin-roofed neural tube full of cerebro-spinal fluid under moderate pressure), are dependent on factors which are not present in the spinal cord of *Polistotrema*. The extreme caudal end of the roof plate of the fourth ventricle in the higher vertebrates, however, has not been affected by these later factors, and presents a condition where comparison is made possible. From a comparison of the roof plate expansion in the 23 mm. human embryo (figs. 26 and 27, *R.Ex.*), in the 15 mm. human embryo (figs. 28 and 29, *R.Ex.*), and in the 8 mm. human embryo (fig. 31, *R.Ex.*) with the second roof plate expansion of the spinal cord in the 20 cm. *Polistotrema* series (figs. 4 and 5, *R.Ex.* 2, and figs. 15 to 17, *R.P.Ex.* 2) and with the third roof plate expansion of the same series (figs. 4 and 5, *R.Ex.* 3), it is evident that the same main factor is present in all, namely, a migration outward and upward of certain roof plate ependymal cells to form an enlarged dorsal cavity. In each case the purpose of this structure is to form an organ for the production and storing of cerebro-spinal fluid. As soon as these structures assumed the function of infiltrating and secretory organs their walls became further expanded from internal pressure of the cerebro-spinal fluid.

From a review of the early stages of the formation of the roof expansion in man, the pig, *Amblystoma*, *Squalus*, and *Petromyzon*, it is fair to assume that the source and early development of the roof expansion of the medulla are identical to the three similar roof plate expansions, described for the spinal cord of a 20 cm. *Polistotrema*. It is possible that both had their *phylogenetic anlage* as mutations, the former from some primitive vertebrate, and the latter from a normal *Polistotrema*, that both were useful and dominant characters, and in case of the medulla, where the animal was allowed to reproduce, this character became preserved for the race.

CAUSES UNDERLYING THE FLATTENING OF THE SPINAL
CORD IN CYCLOSTOMES

If our attention is first directed to a transverse section through an adult *Polistotrema* (figs. 10 and 71) it might be inferred, since there is ample room in the membranous neural canal for a well-rounded spinal cord, that the flattening of the spinal cord might be attributed entirely to internal factors. A glance at a transverse section through a developing *Polistotrema* spinal cord (fig. 57) suffices to show that there is proportionately much less room within the membranous neural canal, and that a mechanical force in the form of a rapidly growing notochord is at work immediately below the spinal cord.

At the outset it seems advisable to establish arbitrarily a typical state of an embryonic spinal cord, by which a direct comparison of one form can be made with another. The examination of very early stages of the spinal cord in a large number of embryos of *Squalus*, *Amblystoma*, the chick, and the pig, in all of which the neural tube is formed by the rolling up of the neural plate, shows that the neural tube passes through three stages: a) A depressed tube with the central canal in the form of a horizontal cleft; b) a cylindrical tube with the canal circular in cross section; and c) a laterally compressed tube with the canal in the form of a vertical cleft (figs. 81 to 86). The existence of this series of changes in *Squalus* has been shown in a table of developmental stages compiled by Scammon. The third stage may be selected as the typical embryonic spinal cord. This stage is reached at about the time of the first appearance of nerve fibers in the marginal layer.

As a result of a comparison of the typical embryonic stages of the spinal cord in the following transverse sections³ (fig. 39 for *Petromyzon*, fig. 55 for *Polistotrema*, fig. 81 for *Squalus*, fig. 83 for *Amblystoma*, fig. 84 for the turtle, fig. 85 for the chick, and fig. 86 for the pig) it is clear that we have all gradations from

³ It is obvious that this comparison would have no value unless the sections were truly transverse sections. To avoid selecting oblique transverse sections, these figures were always drawn from anterior trunk sections if the embryo showed any flexures.

the nearly cylindrical spinal cords of the Cyclostomes (Petro-myzon and Polistotrema) to the very much compressed (flattened laterally) cords of the chick and pig. Were it not for the intermediate stages of Amblystoma and the turtle we might be justified in establishing two distinct types of the embryonic spinal cord: type 1 cylindrical, and type 2 compressed. We could even go further and classify the Cyclostome embryonic cord under type 1 and the Gnathostome cord under type 2.

While the equal expansion of the spinal cord through the addition of nerve cells and fibers to the typical embryonic stage in the pig would tend to produce a cylindrical spinal cord, and in the Cyclostome would tend to produce a depressed cord, the internal structure shows that the origin of the differences between the spinal cord of a Cyclostome and a mammal is not so simple. The neural axis of Petromyzon in an early stage, corresponding to stage (a) above, is decidedly compressed instead of depressed. The problem then is to explain the change from a laterally compressed cord in the early Petromyzon embryos to the gradually depressed, ribbon-like, spinal cord of the adult.

A careful examination of figures 55 to 57, which are taken from practically the same region from three different Polistotrema embryos, shows very clearly that the growing notochord is bringing about the marked flattening (depression) and ventral indenting of the spinal cord. In connection with figure 55 it should be noted that the spinal cord is in the so-called typical embryonic stage, and amid surroundings peculiarly favorable for undergoing a depression from a rapidly growing notochord. It will be seen that the spinal cord is closely enveloped by a meningeal membrane (*P.M.*), approximating a layer of connective tissue, the future membranous neural arch, which is firmly attached below to the growing notochord. Directly above, the mesenchyme is proliferating and migrating toward the center to form a median dorsal cartilage (*M.D.C.*); while there is apparently but little lateral resistance in the way of massing of mesenchyme and the formation of myotomes. In a somewhat later stage (fig. 56) some growth has taken place in the notochord, producing a slight indentation on the spinal cord. In a much

later stage (fig. 57) a median dorsal cartilage has been formed. The much stronger membranous neural arches are firmly attached above to this dorsal cartilage and below to the notochord. The soft plastic spinal cord is thus closely confined by the dorsal cartilage above and less closely by the neural arches laterally. The notochord beneath it has grown very rapidly and its enormous increase in size has brought about a decided flattening (depression) of the spinal cord and indentation of its ventral surface.

It is equally clear in *Petromyzon* also that the growing notochord is to be looked upon as a direct cause for bringing about the flattening of the spinal cord. The external conditions surrounding the spinal cord are shown in figure 58 to be equally favorable for assisting the notochord in this process, with the possible exception that the membranous neural arch is attached above to a membranous neural spine instead of a cartilage, which, however, may be compensated for by an increased dorsal growth of the myotomes.

The hypothesis that the flattening of the spinal cord in *Cyclostomes* is largely brought about by the upward growth of the notochord after the manner set forth in the previous paragraph is considerably strengthened by the fact that a certain relationship exists between the size of the notochord and the amount of flattening of the spinal cord.

This is clear in the 20 cm. *Polistotrema* series from sections of the posterior end of the spinal cord (figs. 19, 21, and 22) and from the photographs of the model of the same region (figs. 7 to 9); the flattening (depression) of the spinal cord becoming less evident as the notochord decreases in size. More striking is a similar relationship shown in the tail region of the 70 mm. *Polistotrema* (figs. 59 and 60) for the reason that these two sections were only one-fourth of a millimeter apart. The above relationship between the size of the notochord and the depression of the spinal cord can be demonstrated fully as conclusively in the medulla region (see figs. 61 and 62). It should be recorded for these two sections that their structure is the same as that of the spinal cord and that they are less than one-half

a millimeter apart. Also the same relationship could be shown in sections anterior to figure 62, and from similar sections of a *Petromyzon* larva. A possible objection to applying this argument in the tail region might arise from the fact that the extreme posterior end of the spinal cord is non-nervous, consisting entirely of ependyma and undifferentiated embryonic cells. In reply to this we would invite comparison of figures 21 and 22, where the structure is non-nervous in both cases, and where the effect of the notochord is obvious.

That the spinal cord of the higher vertebrates has not been depressed by pressure from the notochord is due obviously to the fact that the notochord is an embryonic structure, which never attains sufficient size to have any influence on the spinal cord. This is clear from figures 85 and 86. In Cyclostomes, however, the notochord is a very important structure, develops early and grows for a long period of time, and serves as the skeletal axis of the adult. In fishes, Amphibia, and reptiles the growing notochord may have some slight effect in flattening of the adult spinal cord. In *Amphioxus* the ventral surface of the spinal cord clearly shows the indenting effect of the growing notochord (fig. 70), and in the trunk region where the diameter of the notochord is greatest, the spinal cord is most depressed.

GENERAL CONSIDERATIONS AND SUMMARY

From the foregoing facts the following conclusions seem fully warranted:

1) In the development of the roof plate expansion (tela chorioidea) in the medulla oblongata of most vertebrates three separate stages or epochs of expansion should be recognized: a) A first enlargement of the dorsal portion of the embryonic central canal took place from a migration outward and upward of certain of the roof plate cells, or as was the case in *Petromyzon* from the migration of the nuclei and probable disintegration of the cytoplasm. b) The second stage in the expansion of the roof plate was the direct result of an increase of pressure from the cerebro-spinal fluid, produced from at least two possible

sources. First in the lower vertebrates, as a consequence of considerable embryonic cerebro-spinal fluid being forced into the fourth ventricle from the closure of the dorsal and central portions of the embryonic central canal in the spinal cord and the ventral portion of the embryonic central canal in the medulla, through the union and fusion of the corresponding portions of the lateral plates. Second, in all vertebrates, through the production of cerebro-spinal fluid by the walls of the fourth ventricle assuming the rôle of infiltration, diffusion and secretion. That the ventral portion of the embryonic central canal of the spinal cord was not expanded by the same cause is explained by the fact that it was reinforced at a very early stage by nerve fibers and supported by a growing notochord. c) A third and final stage in the expansion of the roof plate in the higher vertebrates was brought about by the appearance of a pontine flexure acting on a thin-roofed medulla filled with cerebro-spinal fluid, itself under moderate pressure.

2) It is the opinion of the writer that the most important factors in bringing about the expansion of the roof plate of the medulla are those concerned in the second stage described above. For a considerable period of time these factors apparently work in conjunction with the forces of the first stage in resisting counter ingrowths of the lateral plates which would tend to close up the central canal. Since it was shown that no pontine flexure occurred in *Petromyzon*, it can be concluded that the rather extensive expansion of the roof plate in the medulla of this genus was accomplished solely through the factors entering into the first and second stages. For the second stage it was recorded in most cases, especially well shown in the pig, that the size of the fourth ventricle and the expansion of its roof plate bear a close relationship to the amount of coagulum seen in sections. Since this coagulum did not appear in sections until after the roof plate had attained the function of producing cerebro-spinal fluid, it probably indicates a chemical change in the cerebro-spinal fluid, the products of secretion now being added to the tissue fluids which entered the ventricle by infiltration. As a product of secretion, the cerebro-spinal fluid would be capable of exerting

considerable pressure on the thin and plastic roof plate. The tela chorioidea is differentiated as an organ for the production of cerebro-spinal fluid before blood vessels have entered the central nervous system, at a time when the nutritive function of this fluid is important.

3) The appearance of a third stage in the roof expansion of the medulla, due to a pontine flexure, is of little significance save in the higher vertebrates, where it was held that without cerebro-spinal fluid confined in the ventricle there would be no reason for maintaining that a further expansion of the roof plate would take place from the action of a pontine flexure; more than likely, the roof plate would have been folded up within the ventricle. In His' experiment with the bending upward of a dorsally-slit piece of rubber tubing, the elasticity of the rubber tubing, which forced apart the cut surfaces, would be comparable to the action of the cerebro-spinal fluid under moderate pressure within the ventricle, which factor His has apparently disregarded.

4) In the adult *Amphioxus* there is nothing which for a certainty could be homologized to the fourth ventricle and its expanded roof plate. Two isolated cavities in the region of the roof plate, which might be taken for the anlage of the fourth ventricle, appear to the writer to be nothing more than vestiges of a much larger embryonic central canal. If *Amphioxus* possesses no fourth ventricle in the adult we may safely conjecture that more primitive vertebrates had a central nervous system in which there was no distinction between medulla and spinal cord.

5) In an attempt to trace the phylogenetic history of the roof expansion of the fourth ventricle in living vertebrates, the peculiar modification of the fourth ventricle in the adult *Polistotrema* (*Bdellostoma*) should be recorded here even though it has been accurately described by Sanders, Holm, Miss Worthington, Sterzi, Cole, and Nicholls. From the adult it is evident that the well-formed fourth ventricle of the embryo has become transformed through a process of centralization to a deep-seated canal, for the most part no larger than the central canal of the spinal cord. Of especial interest is the fact that its anterior and pos-

terior portions has developed into specialized organs for the production of cerebro-spinal fluid. Notwithstanding this specialization, the fourth ventricle is thought to be decidedly inferior to the tela chorioidea of *Petromyzon* as an organ for the production of cerebro-spinal fluid.

6) In the spinal cord of one individual of *Polistotrema* there occurred at least three expansions of the roof plate which resemble the roof of the fourth ventricle in other vertebrates. From the fact that these expansions were very vascular and their cells granular it is inferred that they functioned as choroid plexuses for the formation of cerebro-spinal fluid. The writer presents the hypothesis that the fourth ventricle in ancestral vertebrates may have originated as a mutation, similar to this sport plexus in the spinal cord of *Polistotrema*; that such sport expansions may have occurred at various places such as the diencephalic segment, the roof of the mesencephalon where a choroid plexus still exists in *Petromyzon*, and in the hind brain and spinal cord. Such mutations, proving to be useful have been preserved in the vertebrate race.

Concerning the flattening of the spinal cord in Cyclostomes

7) A great variation in the shape of the so-called typical embryonic spinal cord is to be recorded. In *Petromyzon* it was found to be nearly cylindrical, to be moderately compressed in *Squalus*, *Amblystoma*, and in the turtle, and decidedly compressed in the chick and the pig.

8) To obtain this typical stage the original compressed spinal cord of *Petromyzon* must have undergone a marked depression, and the early depressed neural tubes of *Squalus*, *Amblystoma*, turtle, chick, and pig must have undergone a decided compression. The main factor causing this depression in the former was thought to be ventral pressure from a growing notochord, and the compression of the latter was attributed to lateral pressure from the growing myotomes.

9) Transverse sections immediately before and during the time that the greatest depression of the spinal cord is taking place

in *Polistotrema* and *Petromyzon* show conclusively that the main factor involved is the pronounced growth of the notochord. It was further established that the embryonic spinal cord was not only in a very plastic condition, but that the general environment was decidedly favorable for bringing about a depression of the spinal cord through this agency.

10) The conclusions outlined in 9) were considerably strengthened by the fact that a direct relationship was established, in both the medulla and tail region, between the size of the notochord and the amount of depression exhibited in the spinal cord. This was shown in late embryos in both *Polistotrema* and *Petromyzon*, and in adult *Polistotrema*.

11) That a similar depression did not take place in the higher vertebrates from a growing notochord was explained by the fact that the notochord is relatively a transitory and insignificant structure; while in the Cyclostomes it is not only formed early in embryonic life, but grows rapidly and continuously.

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PLATE 1

EXPLANATION OF FIGURES

1 to 9 represent photographs from models of the extreme posterior end of the spinal cord from a 20 cm. *Polistotrema* (*Bdellostoma*), illustrating a series of three extensions of the central canal into roof plate expansions. These models were prepared at a magnification of 100 diameters, and were reduced one-half in photographing. In a few cases where certain outlines were somewhat indistinct in the photographs they were strengthened with pen and ink. The roof plate expansions in these models were painted lighter and appear the same in the photographs. Except at the posterior end of the last model, all of the models of the spinal cord exhibit a marked depression and in ventral views they show a pronounced indentation at the center immediately above the notochord. As indicated by an arrow, the caudal direction in figures 1 to 3 is toward the left; while in figures 4 to 9 it is toward the right.

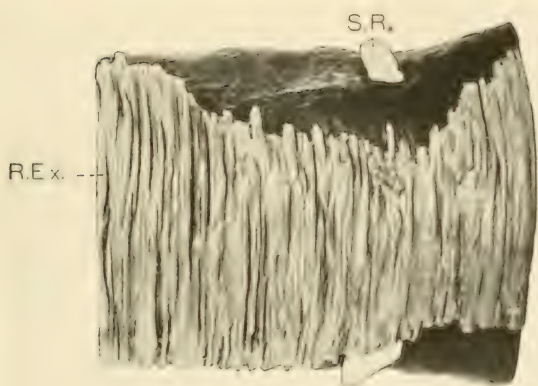
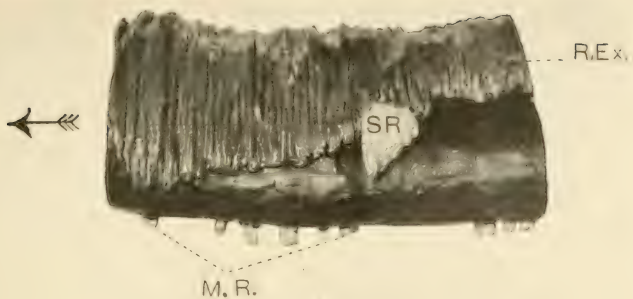
1 The most anterior of the four models seen from the right side. It includes a distance of about two segments. Observe that the roof plate expansion, which covers a large portion of the dorsal surface of the spinal cord, is rather low. It is not known where it begins, or whether there are other outcroppings in front of it as there are behind. It extends caudad some little distance on this model. On the dorsal surface of the cord one dorsal or sensory root is shown, and on the ventral side one ventral root is seen in entirety, being composed of several rootlets. $\times 50$.

2 Dorsal view of the same model shown in figure 1, seen from above. Note that the roof expansion covers a large portion of the spinal cord. At the anterior end it occupies a large central portion, then becomes gradually smaller, and at the same time is confined largely to the left side, after which it gradually increases in size until the posterior end of the model is reached, where it is decidedly wider than at the anterior end, and extends farther over to the right side than to the left. $\times 50$.

3 Ventral view of the cast of the central canal and extension of the same up and out into the roof expansion of the same region of the cord as is shown in figures 1 and 2. It will be seen that the central canal and cavity of the roof plate expansion are connected throughout. In certain places on the right side, shown in white, the roof plate is solid, consisting of ependymal and connective tissue in place of a cavity. For some distance posteriorly on the right side, where the roof expansion is widest, there is no cavity in the roof expansion. The knob-like projections from the right side of the cast of the central canal represent diverticula, and in sections through such a region there would appear to be two central canals. $\times 50$.

ABBREVIATIONS

<i>C.C.</i> , central canal or cast of the same	<i>M.R.</i> , motor or ventral spinal nerve root
<i>C.C.Ex.</i> , central canal extension into roof plate expansion or cast of the same	<i>R.Ex.</i> , roof plate expansion
	<i>S.R.</i> , sensory or dorsal spinal nerve root



2



3

PLATE 2

EXPLANATION OF FIGURES

4 Lateral view of the left side of the second model of the *Polistotrema* spinal cord, which should follow figure 1. Observe the continuation of the first roof plate expansion noted in figure 1 and two additional outcroppings of the roof plate (*R. Ex. 2* and *3*). Between these outcroppings the spinal cord is perfectly normal. Three motor and sensory roots are shown in the figure. $\times 50$.

5 Dorsal photograph of the same model as figure 4. The extent and positions of the three roof plate outcroppings previously mentioned in the description of figure 4 are well portrayed here, as are also the sensory roots. $\times 50$.

6 Dorsal view of a cast of the more caudal portion of the central canal and cavity of the first roof plate expansion. This cast differs from the more anterior cast in that there are no connections between the central canal and the cavity of the roof expansion. Evidence from transverse sections favors the view that a connection once existed, which has been cut off in later development. In the two posterior outcroppings of the roof plate there is a connection between the central canal and the cavity of the roof expansion. The light places in the photograph are indicative of places in the roof expansion where it is solid and contains no cavity. They are more numerous and decidedly larger than was shown in the more anterior model (fig. 3). $\times 50$.

Between models 2 and 4 (figs. 4 and 7) comes model 3, the photograph of which has not been included as a figure. It is about equal in length to models 2 and 4, simply connecting the two, without presenting any peculiarities in roof plate expansion, etc.

ABBREVIATIONS

<i>M.R.</i> , motor or ventral spinal nerve root	<i>R.Ex.(3)</i> , third roof plate expansion in the <i>Polistotrema</i> cord
<i>R.Ex.</i> , roof plate expansion	<i>S.R.</i> , sensory or dorsal spinal nerve root
<i>R.Ex.(2)</i> , second roof plate expansion in the <i>Polistotrema</i> cord	

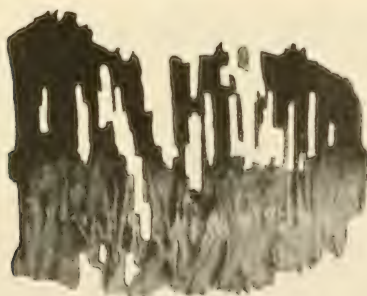
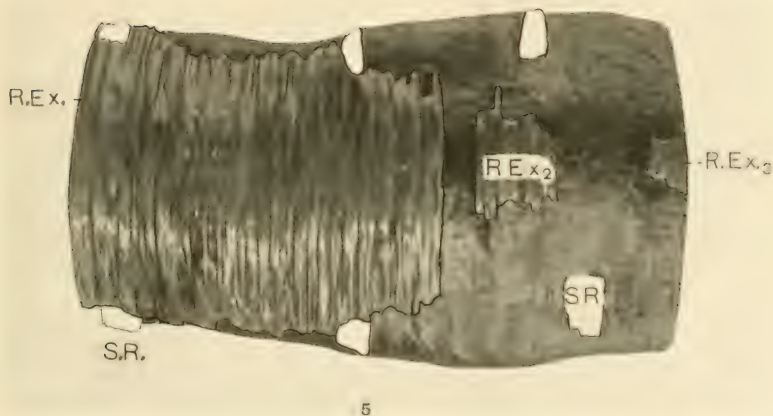
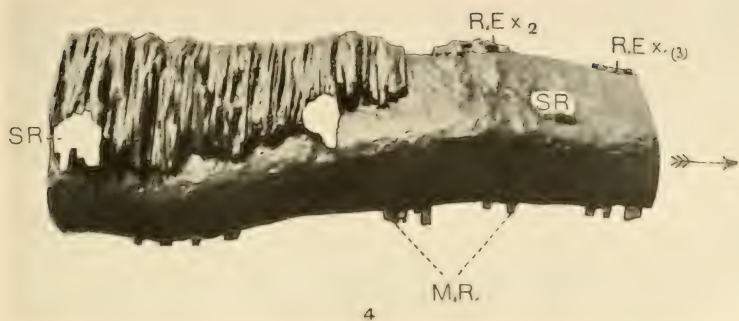


PLATE 3

EXPLANATION OF FIGURES

7, 8, and 9 represent lateral, dorsal, and ventral photographs of the fourth model, which includes the extreme posterior end of the spinal cord. Note especially the swelling above and below (*S.T.*) caused probably by an abnormal sinus terminalis. From this point caudad two factors occur, which may greatly modify the shape of the spinal cord. First, the notochord gradually decreases in caliber and ends at (*Nc. 1*); second, the spinal cord has not developed a nervous structure, consisting solely of ependyma and round undifferentiated embryonic cells. As a result the spinal cord will be seen to gradually become rounded, and after extending past the notochord it ends in dorsal and ventral processes that become lost in the surrounding connective tissue. The posterior motor roots exhibit a reduction in the number of rootlets, and they approximate each other more closely. The posterior sensory roots become greatly reduced in size, and the last left one has no corresponding roots on the opposite side. $\times 50$.

ABBREVIATIONS

<i>M.R.</i> (1), last motor or ventral spinal nerve root	<i>S.R.</i> (1), last sensory or dorsal spinal nerve root
<i>Nc.</i> (1), posterior end of Polistotrema notochord shown in model	<i>S.T.</i> , sinus terminalis

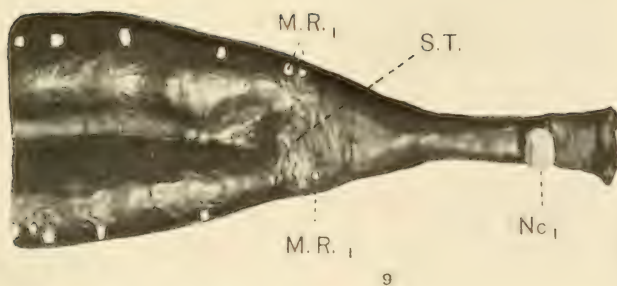
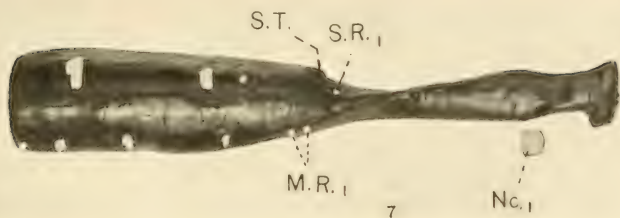
PLATE 4

EXPLANATION OF FIGURES

10 to 23, show 14 transverse sections through the posterior spinal cord region of a 20 cm. Polistotrema (*Bdellostoma*), the same as was modelled and shown in figures 1 to 9. The outlines were all drawn with the aid of a Leitz-Edinger drawing apparatus, using a magnification of 140 diameters and were reduced one-half in reproduction. They are numbered consecutively from anterior to posterior. Figures 10 to 14 pass through what has been designated as the first roof plate expansion; figures 15 to 17 through the second roof plate expansion; figure 20 through the abnormal sinus terminalis; and figures 21 to 23 at various intervals through the extreme posterior end of the spinal cord, which has failed to develop any nervous structures, and has not to any extent been flattened in its development by the growth of the notochord. The enormous concavity seen on the ventral surface of spinal cord in figures 11 to 14 is to a large extent an artifact due to the preparation of the series. It should be noted that the cavities of the roof plate expansions are full of a fibrillar feltwork, for the most part coagulated cerebro-spinal fluid, that their walls are moderately expanded by it, and their cells are sufficiently granular to suggest a secretory function.

10 is from the most anterior section of this series. The entire neural arch, median dorsal cartilaginous bar, and a portion of the notochord are included in this figure. The so-called first roof plate expansion covers a large area of the central portion of the spinal cord; its cavity is in communication with the central canal. The ependymal walls of both the cavity and central canal are composed of several layers of cells. Motor roots, motor cells, substantia gelatinosa cells, and blood vessels are to be seen in transverse section. $\times 70$.

(Continued on page 50)



11 A transverse section taken 840 microns behind figure 10. It would pass through about the center of the first model (figs. 1 and 2). Note that the roof expansion, while containing an enormous cavity, is confined almost entirely to the right side. It is decidedly suggestive of a chorioid plexus forming cerebro-spinal fluid. A blood vessel lies in a fold in its wall and about this fold there is a great mass of coagulum. Throughout the first roof plate expansion, the cavity, and in many places the central canal itself, is larger than either the third or the fourth ventricle. $\times 70$.

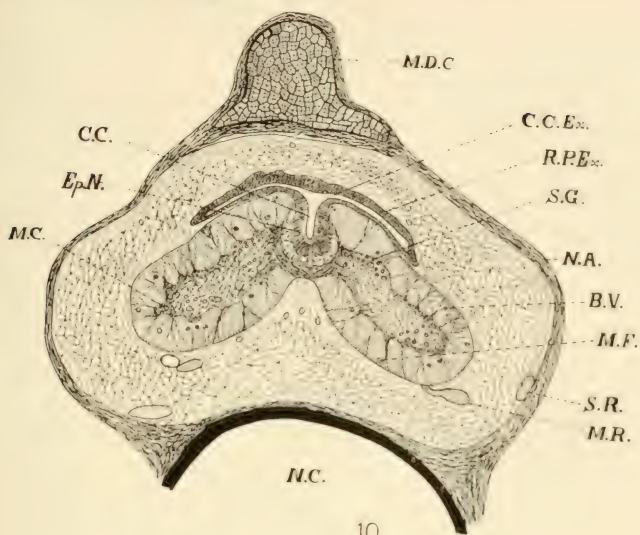
12 From a section 645 microns behind figure 11, and not far from the caudal end of the first model (figs. 1 and 2). At this point the first roof plate expansion and its enclosed cavity attain their greatest width, which cavity is broadly connected with the central canal. $\times 70$.

13 Transverse section 80 microns behind figure 12 and at the very beginning of model 2 (figs. 4 and 5). The dorsal wall of the first roof expansion is very wide and extremely vascular, and the convexity of its walls and the arch of the cavity bear evidence of moderate internal pressure from cerebro-spinal fluid. The wall, while still quite thick, contains a lesser number of ependymal cells, but more connective tissue. Note especially the absence of any direct connection between the cavity of the roof expansion and the central canal. A chain of ependymal cells still connects the two, and may be indicative of a former embryonic connection that has been lost. From this region, caudad, there is no communication between the cavity of the roof expansion and the central canal. That a former connection occurred may be indicated by the fact that at various intervals ependymal cells are scattered between the two. A sensory root can be seen sending its fibers inward toward what is believed to be the substantia gelatinosa. $\times 70$.

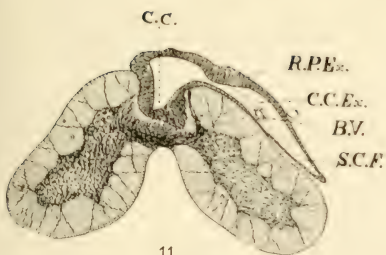
13 A, represents a small portion of the first roof plate expansion and its enclosed cavity from a section taken 410 microns behind figure 13. Observe especially the rich blood supply for the dorsal wall and the ease by which diffusion could take place between a blood vessel and the cavity. A large roof plate cell is drawn separately, highly magnified, directly to the left of 13 A. Note the fine granules in cytoplasm, which gives evidence of being secretory. $\times 125$.

ABBREVIATIONS

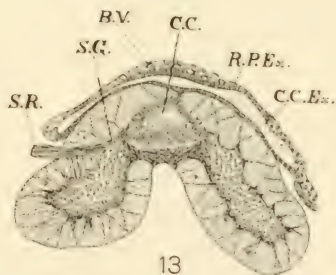
<i>B.V.</i> , blood vessel	<i>M.R.</i> , motor or ventral spinal nerve root
<i>C.C.</i> , central canal	
<i>C.C.Ex.</i> , central canal extension into roof plate expansion	<i>N.A.</i> , membranous neural arch
<i>Ep.N.</i> , layer of ependymal nuclei	<i>N.C.</i> , notochord
<i>M.C.</i> , motor or effective cells	<i>R.P.Ex.</i> , roof plate expansion
<i>M.D.C.</i> , median dorsal cartilaginous bar	<i>S.C.F.</i> , cerebro-spinal fluid
<i>M.F.</i> , Müllerian or giant fiber	<i>S.G.</i> , substantia gelatinosa
	<i>S.R.</i> , sensory or dorsal spinal nerve root



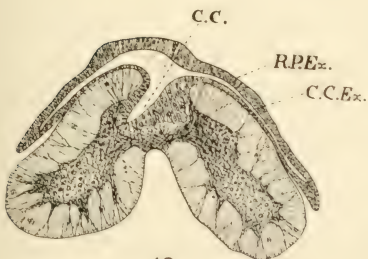
10



11



13



12



13A

PLATE 5

EXPLANATION OF FIGURES

14 From a transverse section taken 19 microns behind figure 13A. It represents a condition found throughout the entire posterior end of the first roof expansion, where a series of cavities are connected with one another. A motor root is seen issuing from the cord. $\times 70$.

15 to 17 Three transverse sections, anterior, middle, and posterior through what has been designated as the second outcropping of the roof plate, which in model 2 (figs. 4 and 5) appears immediately behind the first roof expansion already figured. In figure 15, which is through the anterior end of this roof plate expansion, the outcropping is one of cells only. In figure 16 a distinct cavity containing coagulium is visible, connected below with the central canal. Observe the vascularity of its walls, of the ependyma surrounding the central canal and the diverticula of the same, which doubtless serve as a modified chorioid plexus for producing cerebro-spinal fluid. In figure 17, which is through the posterior end of this roof expansion, a cavity is still present. Its walls, which are mainly connective tissue, are rich in blood vessels. A few scattered ependymal cells and fibers represent a possible connection with the central canal. A third outcropping of the roof plate has been described, but not figured in transverse section. It is located behind the second expansion, and the fact that no ependymal cells are encountered between the first and the second, and the second and third roof expansions, except immediately surrounding the central canal, favors the view that these separate outcroppings of the roof plate in the adult were never connected in an embryonic state. $\times 70$.

18 and 19 Two sections some distance caudad of figure 17 and some little distance apart. They show the spinal cord to be perfectly normal, but to be gradually tapering down in size. Figure 18 is from that part of the cord represented by model 3. The ependymal area in the center has maintained its original size, the reduction that has occurred in size is to be found in the nervous portion. Not only does the ependymal area broadly divide the spinal cord into two halves, but it has here as elsewhere in a number of places, entirely obliterated the central canal for a space of a few microns. Figure 19 is some distance caudad of figure 18, passing through the sensory nerve roots one segment anterior to the abnormal sinus terminalis (model 4, fig. 9, *S.T.*). The spinal cord will be seen to be fast losing its nervous structure, for no motor cells will be seen in this section or in any further caudad. $\times 70$.

ABBREVIATIONS

<i>B.V.</i> , blood vessel	<i>M.V.C.</i> , median ventral cartilaginous bar
<i>C.C.</i> , central canal	<i>N.A.</i> , membranous neural arch
<i>C.C.Ex.</i> , central canal extension into roof plate expansion or east of the same	<i>Nc.</i> , notochord
<i>Ep.C.</i> , ependymal cells	<i>R.P.Ex.</i> , roof plate expansion
<i>Ep.N.</i> , layer of ependymal nuclei	<i>R.P.Ex. (2)</i> , second roof plate expansion
<i>M.D.C.</i> , median dorsal cartilaginous bar	<i>S.G.</i> , substantia gelatinosa
<i>M.R.</i> , motor or ventral spinal nerve root	<i>S.R.</i> , sensory or dorsal spinal nerve root

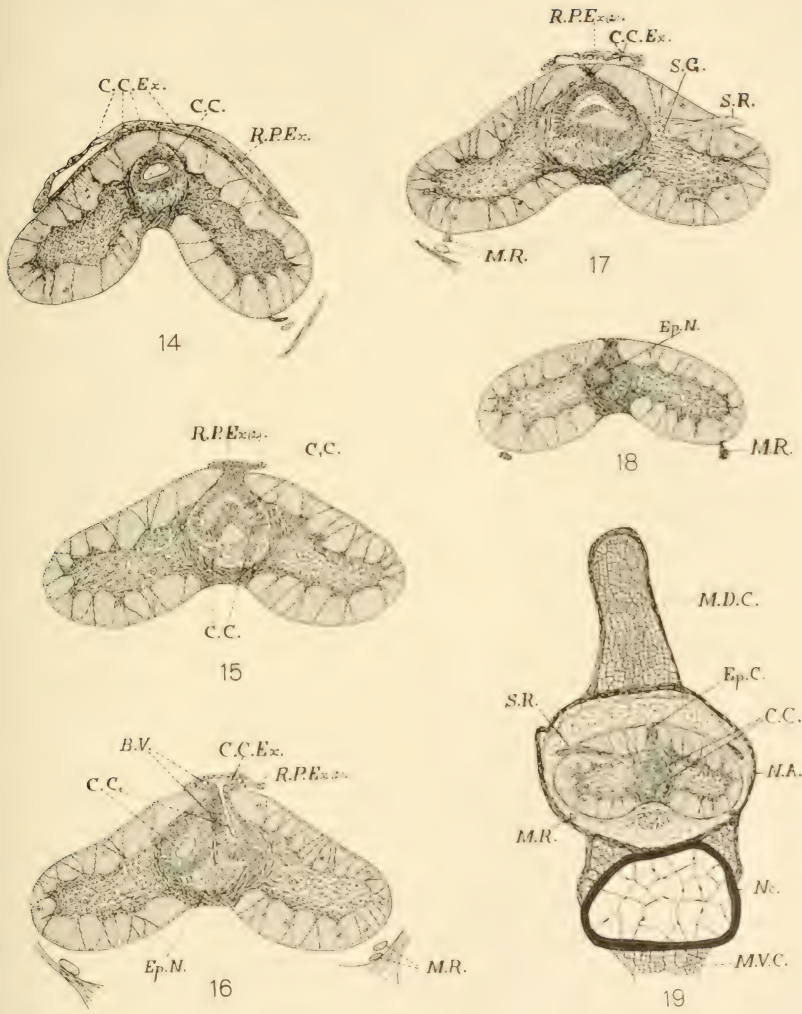


PLATE 6

EXPLANATION OF FIGURES

20 Through the center of the abnormal sinus terminalis, showing the last pair of sensory roots (not last sensory root) in section. Observe especially the large abnormal sinus together with the rich vascular supply for the tissue immediately surrounding it. Note also the dorsal and ventral swelling caused by this sinus and compare with photograph of the model (figs. 7 and 9). They show every evidence of being expanded by the cerebro-spinal fluid. From this point caudad no nervous structures have developed in the spinal cord. Also from this point caudad the notochord gradually decreases in caliber. $\times 70$.

21 to 23 represent three transverse sections taken through the extreme posterior, non-nervous, end of the spinal cord, which is composed solely of supporting tissue and undifferentiated embryonic cells. Figure 21 is the most cephalic, and passes through the spinal cord a short distance behind the abnormal sinus terminalis (figs. 9 and 20, *S.T.*). The spinal cord is still flattened here, but not indented ventrad, and contains a normal central canal. In figure 22 there has occurred a marked reduction in the size of the notochord. Observe that the spinal cord has not become flattened as it has more anteriorly, where the notochord is massive. With figures 21 and 22 compare figures 59 and 60, which are taken from a similar region of the spinal cord from a 70 mm. *Polistotrema* embryo. Figure 23 is the last of this series of drawings. It passes through the extreme posterior end of the spinal cord, some 45 microns caudad of the last trace of the notochord. Note the presence of a central canal, and that the cord has separated into several processes, which further on become lost in the surrounding connective tissue. $\times 70$.

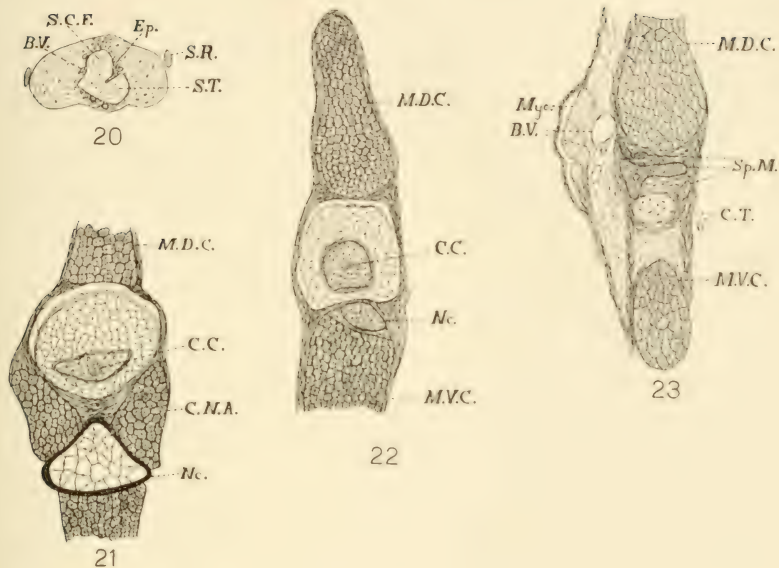
PLATE 7

EXPLANATION OF FIGURES

24 to 31 From transverse frontal sections through the medulla and spinal cord of several human embryos, drawn with the aid of an Edinger-Leitz drawing apparatus, and reduced 3 diameters in reproduction.

24 and 25 From transverse sections through the rhombic brain (frontal through the embryo) of a 23 mm. human embryo; figure 25 passes through the V root and posterior end of the cerebellar rudiments (lateral lobes), while figure 24 is from a more anterior section. The roof expansion (chorioid plexus) is shown as a conspicuous black line in these figures. Everywhere within the boundaries of the roof expansion, the cavity is filled not only with coagulated cerebro-spinal fluid, but with embryonic red corpuscles. Whether these entered through venolymphatic openings (C) or are the result of extravasations was not determined. Wherever mesenchyme borders the roof expansion it is very vascular. It is apparent that the roof expansion is under moderate internal pressure. At first glance the roof expansion will show resemblance to the so-called first roof plate expansion of the spinal cord of the 20 cm. *Polistotrema*, already figured, but its later mode of development was shown to be very different. $\times 10$.

(Continued on page 56)



ABBREVIATIONS

- | | |
|--|---|
| <i>B.V.</i> , blood vessel | <i>Myo.</i> , myotomes |
| <i>C.C.</i> , central canal | <i>Nc.</i> , notochord |
| <i>C.N.A.</i> , cartilaginous neural arch | <i>S.C.F.</i> , cerebro-spinal fluid. |
| <i>C.T.</i> , white fibrous connective tissue | <i>Sp.M.</i> , spinal cord |
| <i>Ep.</i> , ependyma | <i>S.R.</i> , sensory or dorsal spinal nerve root |
| <i>M.D.C.</i> , median dorsal cartilaginous bar | <i>S.T.</i> , sinus terminalis |
| <i>M.V.C.</i> , median ventral cartilaginous bar | |

26 and 27 Represent two transverse sections through the medulla, passing through the posterior end of the fourth ventricle of the same series from which figures 24 and 25 were drawn. Note especially the expansion of the roof plate and compare with the so-called second roof plate expansion of the 20 cm. *Polistotrema* spinal cord (fig. 16). It is questionable whether the openings (C) are artifacts or not. As was noted previously, the mesenchyme outside the roof plate is very vascular and the roof plate has the appearance of being under a moderate degree of internal pressure. $\times 10$.

28 A rather oblique frontal section through the medulla of a 15 mm. human embryo (Inst. of Anat., trans. series, II 23). In this region the contour of the rhombic brain is such that the posterior part of the roof expansion of the fourth ventricle is cut transversely; while the more anterior portion, seen below, appears more or less in frontal section. More anterior sections would show the roof plate to be continuous. The posterior end of the fourth ventricle will admit of direct comparison with the second roof expansion of the 20 cm. *Polistotrema* spinal cord (fig. 16). Had the fourth ventricle been empty, as was the case of the rubber tubing in His' experiments, there would be absolutely no grounds for believing that the anterior portion of the roof plate would be expanded as it is by the appearance of a pontine flexure. It might on the contrary have been folded up within the ventricle. $\times 16.6$.

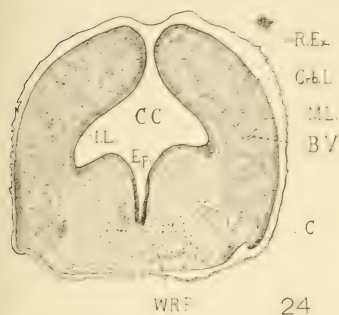
29 A transverse section through the extreme posterior end of the fourth ventricle of the same series as figure 28. There is here a slight roof plate expansion containing no cavity. Compare with figure 15. $\times 16.6$.

30 Transverse section through the thoracic spinal cord, taken from the same series as figure 28. Note that the roof plate consists of ependyma only, while the floor is reinforced by white matter, and even at this late stage if any marked increase in pressure occurred from the cerebro-spinal fluid of this region, an expansion of the roof plate would have been entirely possible. $\times 16.6$.

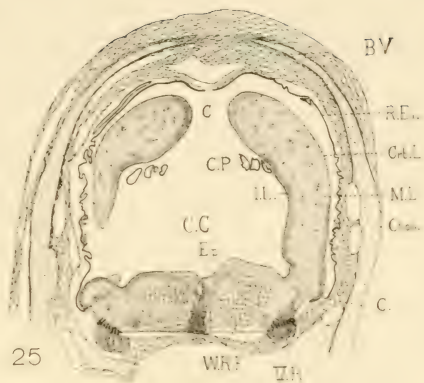
31 Similar to figure 28, but from an 8 mm. human embryo (Inst. of Anat., series H4). In this plane the posterior end of the fourth ventricle is cut nearly transversely, and is directly comparable with the second roof expansion of the 20 cm. *Polistotrema* spinal cord (fig. 16). The cavity was full of coagulum, its walls have the appearance of being under moderate internal pressure, and the adjacent mesenchyme is very vascular. $\times 46.6$.

ABBREVIATIONS

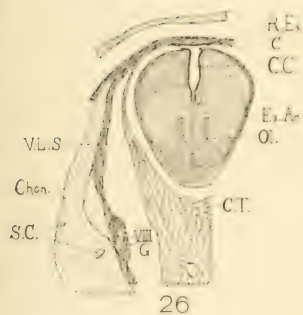
<i>B.V.</i> , blood vessel	<i>Ol.</i> , inferior olive
<i>C.</i> , an apparent communication between the veno-lymphatics and the fourth ventricle	<i>R.Ex.</i> , roof plate expansion
<i>C.C.</i> , central canal	<i>R.L.</i> , rhombic lip
<i>Ch n.</i> , chondrocranium	<i>R.P.</i> , roof plate of the central nervous system
<i>C.P.</i> , choroid plexus of the fourth ventricle	<i>S.C.</i> , semicircular canals
<i>Crb.L.</i> , lateral lobes of the cerebellum	<i>S.R.</i> , sensory or dorsal spinal nerve root
<i>C.T.</i> , white fibrous connective tissue	<i>T.S.</i> , tractus solitarius
<i>Ep.</i> , ependyma	<i>VIII.G.</i> , auditory ganglion
<i>Ex.Ar.</i> , external arcuate fibers	<i>V.L.S.</i> , veno-lymphatic sinus
<i>I.L.</i> , inner or ependymal layer of nuclei	<i>V.R.</i> , trigeminal root
<i>Mar.L.</i> , marginal layer	<i>W.R.F.</i> , white reticular formation
<i>M.L.</i> , mantle layer	<i>XI.R.</i> , accessory nerve root



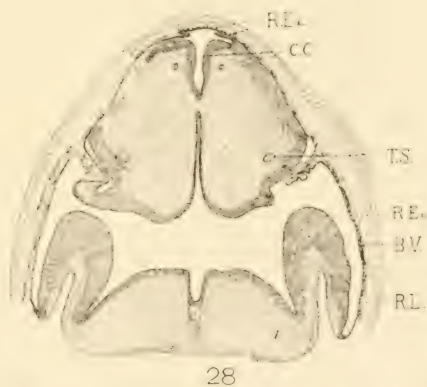
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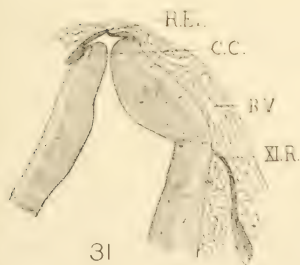
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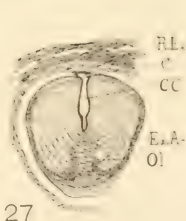
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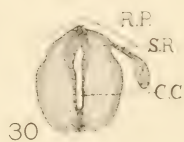
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27



29



30

EXPLANATION OF FIGURES

32 to 53 A series of transverse sections through the region of the V, VIII, and X ganglia in embryos of *Petromyzon* of ages varying from 10 to 26 days. It will be seen from these sections that *Petromyzon* develops an extensive roof expansion without the aid of a pontine flexure, and the cranial and spinal ganglia are well-formed while the central nervous system is a solid cord. All of the figures were drawn with the aid of an Edinger-Leitz drawing apparatus. With figure 54 a magnification of 76.6 diameters was used, while 250 diameters was used for the others. In reproduction they were all reduced one-half.

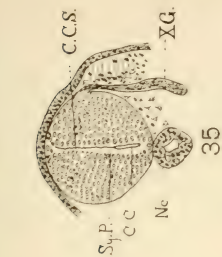
32 Transverse section through the medulla in the region of the auditory vesicle from a 10 day *Petromyzon*. This is my oldest embryo in which the central nervous system has remained a solid cord. Ordinarily it becomes tubular during the seventh day. This section shows the medulla to consist of a syncytium of protoplasm, consisting of a mass of round nuclei, much yolk, and a few fibers in the marginal layer. The nuclei have migrated a short distance to either side of the median dorso-ventral line. A seam (*C.C.S.*) has appeared here, which marks the position and beginning of the embryonic central canal. The protoplasm bordering the central canal seam is finely granular and may be assuming a secretory function. The acoustic ganglia and fibers are shown to be well-differentiated on both sides. $\times 125$.

33 to 35 Three transverse sections passing through the medulla region of another 10 day *Petromyzon* embryo, in which the central canal has been somewhat retarded in development. These sections pass through the V, VIII, and X ganglia respectively, and with the exception that the central canal furrow or seam (*C.C.S.*) has expanded into small dorsal and ventral cavities (no dorsal cavity has appeared in fig. 35) the general structure of the medulla is about the same as in figure 32. Later these cavities will become the dorsal and ventral expansions of the embryonic central canal of the medulla. The protoplasm in the region of this seam is granular and may be secreting an embryonic cerebro-spinal fluid. From these figures it will be seen that the beginning of the central canal occurs at the same time throughout the entire rhombic brain. The anterior portion of the spinal cord, while not figured, contains a central canal furrow in the same stage. $\times 125$.

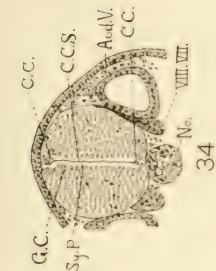
(Continued on page 60)

ABBREVIATIONS

<i>Aud.V.</i> , auditory vesicle or otocyst	<i>Mes.</i> , mesencephalon
<i>Br.A.</i> , branchial arch	<i>M.L.</i> , mantle layer
<i>B.V.</i> , blood vessel	<i>Myo.</i> , myotomes
<i>C.C.</i> , central canal	<i>Nc.</i> , notochord
<i>C.C.C.</i> , central canal closure, caused by fusion of lateral plates	<i>P.B.</i> , pineal body
<i>C.C.S.</i> , central canal seam or furrow, in <i>Petromyzon</i>	<i>R.P.</i> , roof plate of the central nervous system
<i>Ep.N.</i> , layer of ependymal nuclei	<i>Sp.M.</i> , spinal cord
<i>F.L.P.</i> , fused lateral plates of the spinal cord	<i>Sy.P.</i> , syncytium of protoplasm
<i>F.P.</i> , floor plate of the central nervous system;	<i>Tel.</i> , telencephalon
<i>G.C.</i> , germinal cell	<i>V.G.</i> , Gasserian or semilunar ganglion
<i>Mar.L.</i> , marginal layer	<i>VIII.VII.</i> , acustico-fascialis ganglion
	<i>W.M.</i> , white matter
	<i>X.G.</i> , vagus ganglion (nodosum).
	<i>Y.</i> , yolk granules



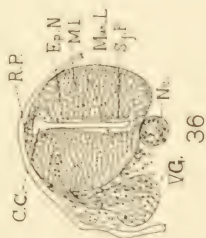
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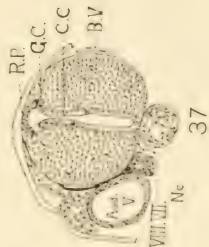
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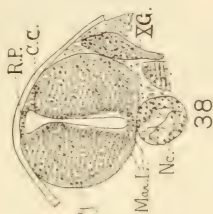
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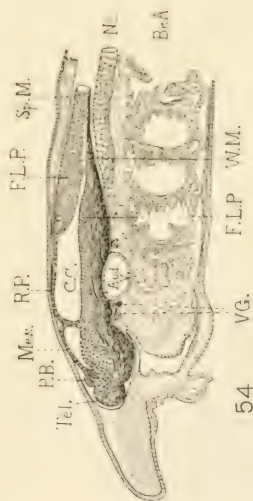
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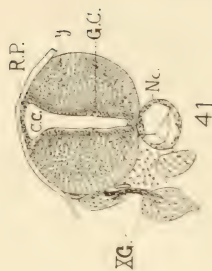
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40

36 to 38 From a 11 day Petromyzon embryo through the same regions as those shown in figures 33 to 35, and to facilitate comparison were placed directly under them. Considerable progress has occurred everywhere. Note 1) that the central canal seam with its small dorsal and ventral cavities in the 10 mm. series has given place to a typical embryonic central canal, which is much wider at the top and bottom than at the center. The constricted portion of course represents the last place for the protoplasm to give way or to be disintegrated. 2) The floor plate is slightly thicker and less expanded than the roof plate, being reinforced on the outside by white matter and by a rapidly growing notochord below. 3) A few more nerve fibers and nuclei have appeared in the lateral plates. 4) The number of dividing germinal cells has increased while the number of yolk granules remains about the same. 5) The first blood vessels have put in appearance directly outside the meningeal membrane (fig. 37, *B.V.* and nearer the roof plate on the opposite side). $\times 125$.

39 Transverse section through the spinal cord of the same 11 day Petromyzon series as figures 36 to 38, showing the so-called typical embryonic spinal cord. $\times 125$.

40 and 41 Somewhat oblique transverse sections from a 12 day Petromyzon, passing in figure 40, through the V ganglion on one side and the VIII ganglion on the opposite side, and in figure 41 through the X ganglion on one side and a region behind the X ganglion on the opposite side. They can readily be compared with the 11 day series above (figs. 36-38). The lateral plates have apparently increased notably in the number of nerve fibers and nuclei, some of which, however, will have to be attributed to the fact that the sections are cut quite obliquely. Also the numbers of nerve fibers have increased in the floor plate. Throughout, the central canal has increased in width. Of especial interest is a small central mass of protoplasm (*Sy.P.*) in figure 40, which for a space of 50 microns persists as the last remnant of a once solid mass of protoplasm in the center of the medulla. It is obvious at this stage that some factor must have produced sufficient internal pressure to prevent the closing up of the ventricle on account of the rapid increase of cells and fibers in the lateral plates. It is fair to assume that this factor is internal pressure from cerebro-spinal fluid. $\times 125$.

54 of this plate will be described in its proper place, opposite the next plate.

PLATE 9

EXPLANATION OF FIGURES

42 to 44 Three transverse sections through the medulla of an 18 day Petromyzon embryo, passing through the V, VIII, and X ganglia, and for the sake of comparison preserving the same order or arrangement as was used for the earlier embryos. A slight increase in the white matter is to be noted for the lateral and ventral plates over the 12 day series; but little, if any change has taken place in the central canal, unless possibly the central portion has increased slightly in width. Absolutely no further expansion of the roof plate has occurred. Since sections through the medulla of a 14 and 16 day series presented about the same appearance as figures 42 to 44 none were figured. $\times 125$.

45 Transverse section through the cephalic portion of the spinal cord from the same series as figures 42 to 44. Observe especially the beginning of the dorsal closure of the central canal (C.C.C.), showing the central canal to consist of dorsal and ventral cavities and a central seam, strongly resembling the stage when it first appeared in the spinal cord. This dorsal closure of the embryonic central canal begins in the anterior portion of the spinal cord much earlier than the corresponding ventral closure of the embryonic central canal in the medulla, occurring in my series of 14 and 15 days. It is obviously caused by the ingrowth of the lateral plates due to the great increase in the number of nerve fibers. $\times 125$.

46 to 48 Three transverse sections through the same region of the medulla of a 20 day Petromyzon as is shown above in figures 42 to 44 for the 18 day embryo. It will be seen that many noticeable changes have taken place, due primarily to a marked increase of nerve fibers in the lateral and ventral plates, and to a slight increase in the number of cells in the lateral plates. The shape of the medulla has become more compressed (flattened out in a dorso-ventral plane). Unquestionably the increase of fibers in the lateral plates has occurred largely in the median and ventral portions. Note the result on the embryonic central canal, which has been completely closed, except for a small dorsal triangular cavity (C.C.) the early fourth ventricle. Its roof plate, however, has expanded somewhat. Observe that true ependymal cells are beginning to take on form about the ventricle, and those in the roof plate may soon assume a secretory function, if they are not already active. Also the blood vessels have become more abundant outside of the medulla, especially in the region of the roof plate, which would make infiltration and diffusion through the roof plate into the ventricle easy. $\times 125$.

49 From a transverse section through the anterior portion of the spinal cord. Observe the great increase in fibers in the lateral and ventral plates, and note that the cleft-like embryonic central canal has become entirely closed, but for a small portion, which will remain as the adult central canal. It is obvious that the two portions of the embryonic central canal which persist in the medulla and the spinal cord are the opposite, being the dorsal in the medulla and the ventral in the cord. $\times 125$.

50 to 53 Four transverse sections through the medulla of a 26 day Petromyzon embryo, taken through the V, VIII, and X ganglia, and through a region behind the fourth ventricle where the central canal is passing ventral to assume its characteristic position in the spinal cord. A comparison with the 20 day series above (figs. 46 to 48) will demonstrate a marked increase in the size of the medulla and the fourth ventricle, and a greater expansion and convexity of the

(Continued on page 62)

roof plate, in consequence of which the dorsal tips of the lateral plates are widely separated. There is a marked increase in the number of nerve fibers in the lateral plates, especially in the median and ventral portions. Of prime importance is the great expansion of the fourth ventricle and the roof plate, which apparently in *Petromyzon* can be explained only from internal factors, the most obvious of which is the mechanical expansion due to an increase in the cerebro-spinal fluid. It will be seen that these forces were sufficiently strong to more than offset the thickening of the lateral plates which would tend to obliterate the dorsal portion of the embryonic central canal as it has the ventral portion. It is apparent that this internal pressure has pushed the lateral wall apart in the dorsal region, where the lateral plates are thinnest and weakest. As was pointed out in the 20 day series the ependymal cells are becoming differentiated and probably have assumed a secretory function. Likewise the increase in the number of blood vessels above the roof plate favors filtration and diffusion into the fourth ventricle. $\times 125$.

54 (See preceding plate.) Median longitudinal section through the head region of a 26 day *Petromyzon* embryo introduced for a comparison with the transverse sections in figures 50 to 53. Note especially that the marked convexity of the roof plate of the fourth ventricle is suggestive of expansion from an increase of cerebro-spinal fluid. Absolutely no pontine flexure is to be seen, the little convexity that occurs in the floor plate can easily be attributed to an increase in the number of nerve fibers. Observe that the fourth ventricle (*C.C.*) is the remains of the dorsal portion of the original embryonic central canal, while the central canal of the spinal cord is the remains of the ventral portion. The ventral portion of the embryonic central canal of the medulla has been obliterated through the fusion of the ventral portions of the lateral plates. $\times 38.3$.

ABBREVIATIONS

<i>Aud.V.</i> , auditory vesicle or otocyst	<i>Myo.</i> , myotomes
<i>B.V.</i> , blood vessel	<i>N.C.</i> , nerve cell
<i>C.C.</i> , central canal	<i>R.Ex.</i> , roof plate expansion
<i>C.C.C.</i> , central canal closure, caused by fusion of lateral plates	<i>R.P.</i> , roof plate of the central nervous system
<i>Ep.</i> , ependyma	<i>V.G.</i> , Gasserian or semilunar ganglion
<i>Ep.N.</i> , layer of ependymal nuclei	<i>VIII.VII.</i> , acustico-fascialis ganglion
<i>G.C.</i> , germinal cell	<i>W.M.</i> , white matter
<i>G.M.</i> , white matter	<i>X.G.</i> , vagus ganglion (nodosum).
<i>M.L.</i> , mantle layer	

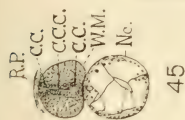
PLATE 10

EXPLANATION OF FIGURES

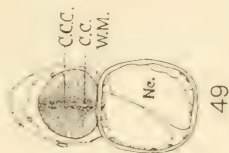
55 to 62 Taken from various transverse sections through embryonic and larval *Polistotrema* and *Entosphenus* (Pacific coast lamprey). Introduced to show the effect of the developing notochord on the spinal cord in Cyclostomes. They were drawn with the aid of an Edinger-Leitz drawing apparatus and reduced one-half in reproduction.

55 Transverse section through the caudal region of a 20 mm. *Polistotrema* embryo. It will be seen at this stage that the notochord has produced very little visible effect on the spinal cord. Cyclostome embryos of this stage (compare fig. 39 for *Petromyzon*) present a nearly cylindrical spinal cord; while that of all other vertebrates is more or less elliptical in cross section, the greater

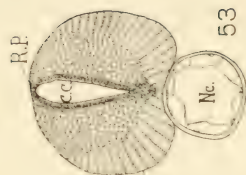
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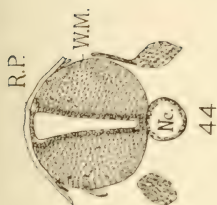
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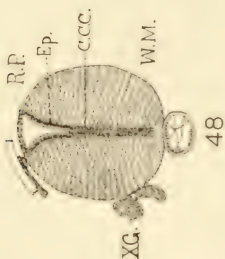
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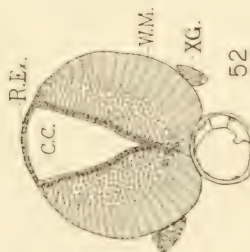
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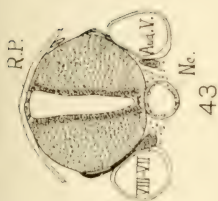
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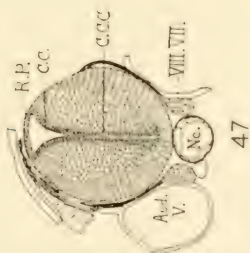
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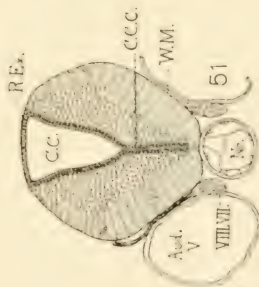
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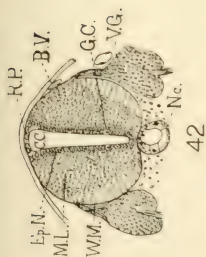
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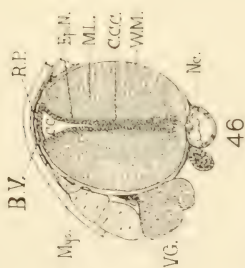
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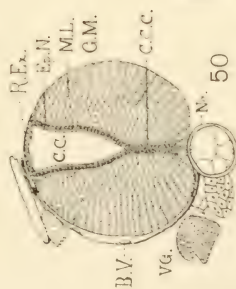
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46



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diameter being dorso-ventral. It should be noted that the spinal cord is enveloped tightly by a meningeal membrane, more or less fused with connective tissue outside that will form the neural arch, which is firmly attached to the notochord below. Immediately above, the mesenchyme is proliferating rapidly and migrating to the center where it will form the median dorsal cartilaginous bar. Little progress has occurred in the formation of the myotomes at the side, and elsewhere there is only loose mesenchyme. $\times 70$.

56 Similar transverse section to figure 55, but from a 27 mm. *Polistotrema* embryo. This slightly later stage shows considerable growth of the notochord and a median indentation on the ventral surface of the spinal cord as the result. Note that the conditions surrounding the development of the notochord previously enumerated under the description of figure 55 are instrumental in assisting the notochord in producing the gradual flattening (depression) of the spinal cord seen in the next figure. $\times 70$.

57 Transverse section of the spinal cord of a 50 mm. *Polistotrema* embryo from the same region as figure 56. It will be seen that the spinal cord is enclosed in a membranous canal of dense connective tissue, attached below to the notochord and above to the median dorsal cartilaginous bar. Above this there are developing cartilaginous rays surrounded by dense connective tissue. The developing myotomes rest against the neural arches both laterally and dorsally. The notochord has increased greatly in size and, pushing up against the soft spinal cord, produces the depression and ventral indentation of the spinal cord exhibited in this figure. It should be noted that the roof plate is still ependyma and an expansion of the roof plate could take place even in this late stage if the mechanical factors enumerated for the medulla of *Petromyzon* were operative here. The thickening of the lateral plates has about obliterated the central portion of the embryonic central canal, leaving only the dorsal and ventral portions, in which there is a fibrillar feltwork, probably representing both cerebro-spinal fluid and ependyma cilia. Reissner's fiber is visible in the ventral or permanent central canal. $\times 70$.

58 Transverse section through the tail region of a 20 mm. *Entosphenus* larva. It will be observed that the spinal cord is further developed than in the 27 mm. *Polistotrema* embryo (fig. 56). It is apparent that the same factors are involved in flattening the spinal cord as were enumerated for *Polistotrema*. The notochord has made fully as much growth and the structures surrounding the spinal cord are the same as in *Polistotrema*, with the exception that instead of a median dorsal cartilage for the attachment of the membranous neural arch there is a membranous neural spine. To some extent this may reduce the dorsal resistance, but on the other hand it may be compensated for by a greater development of the myotomes above the neural arch. $\times 125$.

ABBREVIATIONS

<i>C.A.</i> , caudal artery	<i>M.V.C.</i> , median ventral cartilaginous bar
<i>C.C.</i> , central canal	<i>Myo.</i> , myotomes
<i>C.H.</i> , caudal heart	<i>N.A.</i> , membranous neural arch
<i>C.V.</i> , caudal vein	<i>Nc.</i> , notochord
<i>D.R.</i> , dorsal cartilaginous rays	<i>P.M.</i> , pia mater or meningeal membrane of the younger stages
<i>Ep.N.</i> , layer of ependymal nuclei	<i>R.P.</i> , roof plate of the central nervous system
<i>L.S.</i> , lateral veno-lymphatic sinus or anlage of the same	<i>Sp.G.</i> , spinal ganglion
<i>Mar.L.</i> , marginal layer	<i>V.T.</i> , ventral veno-lymphatic trunk
<i>M.D.C.</i> , median dorsal cartilaginous bar	<i>W.M.</i> , white matter

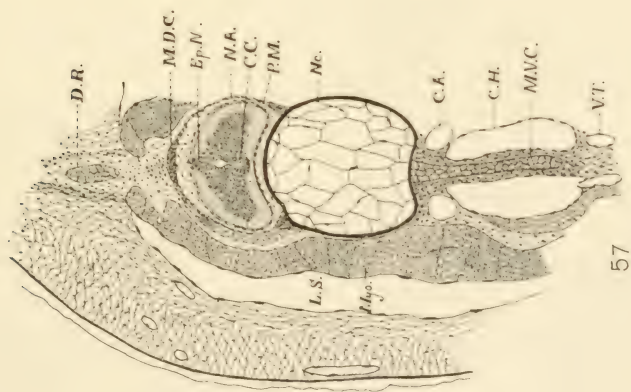
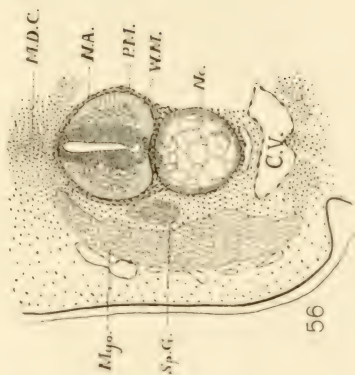
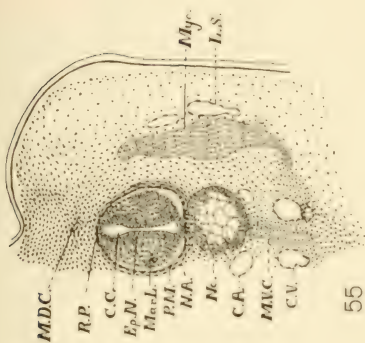
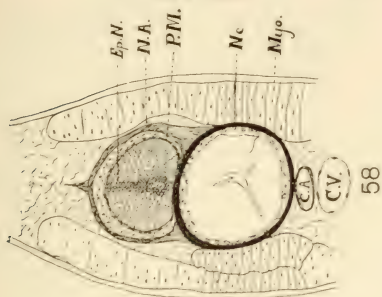


PLATE 11

EXPLANATION OF FIGURES

59 and 60 Two transverse sections only 290 microns apart through the extreme posterior end of the spinal cord of a 70 mm. *Polistotrema* embryo. In the more anterior section (fig. 59) the spinal cord is depressed, contains developing nervous elements, and the notochord is large proportionately. In the posterior section (fig. 60) the diameter of the notochord is much reduced and only supporting elements appear in the spinal cord. As a result no flattening of the spinal cord has taken place in this region. Compare with figures 21 and 22, which are similar sections through an adult *Polistotrema*. $\times 70$.

61 and 62 Taken from two transverse sections 480 microns apart, through the medulla oblongata of an adult *Polistotrema*. In both sections no nervous structures have appeared that were not present in the spinal cord. Note as you pass caudad (figs. 62 to 61) that the medulla becomes flattened ventrally and dorsally in direct proportion to the increase in size of the notochord. This relationship can be shown fully as marked in more anterior sections, and in sections taken from a similar region of larval *Petromyzon*. $\times 25$.

ABBREVIATIONS

<i>Aud.V.</i> , auditory vesicle or otocyst	<i>M.V.C.</i> , median ventral cartilaginous bar
<i>B.V.</i> , blood vessel	<i>Myo.</i> , myotomes
<i>C.C.</i> , central canal	<i>N.A.</i> , membranous neural arch
<i>E.N.</i> , undifferentiated embryonic nuclei	<i>N.C.</i> , nerve cell
<i>Ep.</i> , ependyma	<i>P.M.</i> , pia mater or meningeal membrane of the younger stages
<i>Ep.N.</i> , layer of ependymal nuclei	<i>P.P.</i> , parachordal plate
<i>L.S.</i> , lateral veno-lymphatic sinus or anlage of the same	<i>Sp.G.</i> , spinal ganglion
<i>M.D.C.</i> , median dorsal cartilaginous bar	<i>W.M.</i> , white matter
<i>M.F.</i> , Müllerian or giant fiber	<i>X.G.</i> in figure 62 should be <i>Sp.G.</i>
<i>M.L.</i> , mantle layer	<i>X.N.</i> , vagus nerve

PLATE 12

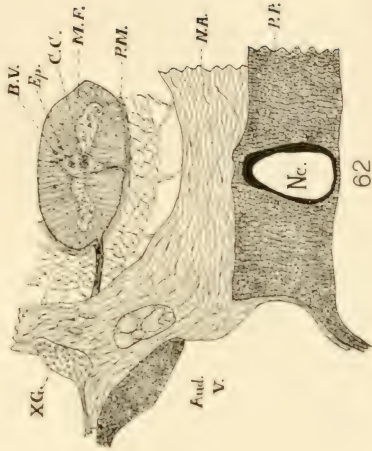
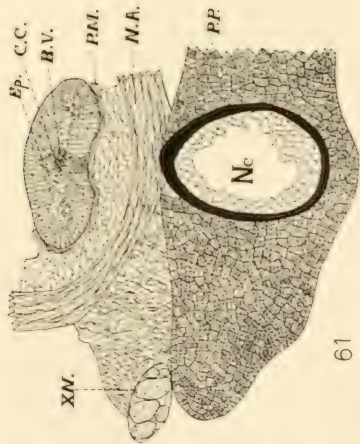
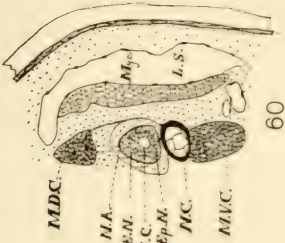
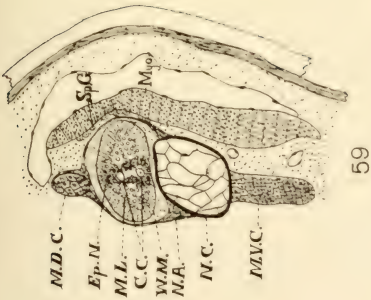
EXPLANATION OF FIGURES

63, represents a diagrammatic reconstruction of the fourth ventricle from an adult *Polistotrema* series, and the planes from which the transverse sections were drawn for figures 64 to 66 are indicated by lines bearing those figures. Observe how the large fourth ventricle of the embryo has been reduced to a small central canal, having a posterior dilation (P_4V), and how the anterior end breaks up into two or more small longitudinal canals that soon terminate in the sinus mesocœlicus.

64 and 65, represent transverse sections, taken at different levels of the fourth ventricle of *Polistotrema*. These sections were drawn with the aid of an Edinger-Leitz drawing apparatus and were reduced one half.

64 From a transverse section through the caudal portion of the mesencephalic lobes (cerebellum of Miss Worthington). Exact plane indicated by line 64 in figure 63. The section passes through the posterior mesocœle or cerebellar ventricle (M'' .) and the sinus mesocœlicus (anterior dilation of the fourth ventricle of Miss Worthington), a short distance behind the branching off of the posterior mesocœle. The cavity contains a fibrillar feltwork, which is in part coagulated cerebro-spinal fluid and in part ependymal cilia. The ependyma surrounding the fourth ventricle is rich in blood vessels, which derives its arterial supply from

(Continued on page 68)



two medulla arteries (*M.A.*). Here as elsewhere, the ependyma surrounding the fourth ventricle doubtless functions as a modified chorioid plexus, discharging cerebro-spinal fluid into the fourth ventricle. It will be seen that the cavity of the fourth ventricle is smaller than the peculiarly modified central canal and roof expansion cavity of the *Polistotrema* spinal cord portrayed in figures 10 to 13. $\times 25$.

65 A more caudal section through the extreme tip of the posterior lobes of the mesencephalon (cerebellum of Miss Worthington), its exact plane being indicated by line 65 in figure 63. It will be seen that the fourth ventricle of the embryo has in this region of the adult become reduced to three small longitudinal canals (*A₄V.*), which are imbedded in a rather large, dense, and vascular ependymal mass. The most dorsal of these canals contains Reissner's fiber. Here again the ependymal walls are probably functional as a modified chorioid plexus. Very shortly these canals reunite and continue some little distance caudad as a small central canal, no larger than the central canal of the spinal cord. $\times 25$.

66 Transverse section through the posterior end of the medulla of the same series as figure 64. The exact plane of the section is indicated by line 66 in figure 63. It passes through what has been designated as the posterior dilation of the fourth ventricle (*P₄V.*), which is nothing more than a fair-sized centrally located cavity, the remains of a much larger embryonic fourth ventricle, surrounded by a great mass of vascular ependyma. The center of this cavity contains a fibrillar feltwork (*S.C.F.*) composed largely of coagulated cerebro-spinal fluid and some ependymal cilia. Here as more anteriorly we probably have a modified chorioid plexus, the ependymal walls and their blood vessels secreting and filtering cerebro-spinal fluid into the fourth ventricle. $\times 25$.

ABBREVIATIONS

<i>A₄V.</i> , anterior fourth ventricle	<i>M.A.</i> , medulla artery
<i>B.V.</i> , blood vessel	<i>Mes'</i> , posterior lobes of the mesencephalon, cerebellum of Miss Worthington
<i>Ep.</i> , ependyma	<i>P₄V.</i> , posterior fourth ventricle
<i>Hab. B.</i> , habenular body	<i>S.C.F.</i> , cerebro-spinal fluid
<i>Inf.</i> , infundibulum	<i>S.M.</i> , sinus mesocælicus
<i>M.</i> , mesocæle or mesencephalic ventricle	<i>V.M.R.</i> , motor V root
<i>M'</i> , anterior portion of the mesocæle or sub-commissural canal of Nicholls	<i>V.</i> , fourth ventricle
<i>M''</i> , dorsal portion of the mesocæle or optocoel and posterior portion of the optocoel of Nicholls	<i>VIII.G.</i> , auditory ganglion
	<i>X.R.</i> , vagus root

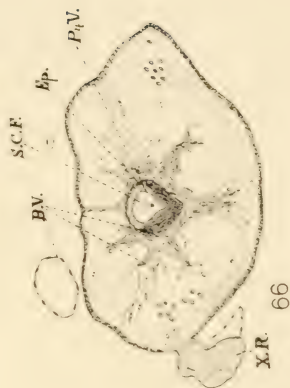
PLATE 13

EXPLANATION OF FIGURES

67 to 69 Three transverse sections through the brain region of a 37 mm. *Amphioxus*. No blood vessels were seen in any of these sections, but the membranous neural canal is surrounded on three sides by enormous veno-lymphatic sinuses, and the structure of the central nervous system is to a considerable extent made up of rather coarse supporting tissue, making infiltration an easy method for nourishing the brain. Drawn with an Edinger-Leitz drawing apparatus and reduced one-half in reproduction.

(Continued on page 70)

WILLIAM F. ALLEN



The most anterior section, figure 67, passes through the anterior ventricle at its highest point, which is a short distance behind the neuropore. This ventricle has no dorsal dilation suggestive of the fourth ventricle. What dilation occurs, is median and ventral. Cilia-like processes from the border of the cells enter the cavity. If the ependymal cells are not secretory it is possible that the cerebro-spinal fluid of *Amphioxus* does not differ from the serum of the adjacent veno-lymphatic sinuses. If nerve cells occur in this region they are small, and in ordinary preparations indistinguishable from ependymal cells. $\times 125$.

68 60 microns behind figure 67. The large central canal of the embryonic brain has evidently become reduced in this region to a ventral central canal (*C.C.*) and a small dorsal isolated cavity (*V.2.*). This isolated dorsal cavity can not be compared with the fourth ventricle of higher vertebrates. It is rather to be looked upon as a vestigial structure, which may aid in the infiltration of lymph from the outer veno-lymphatic sinuses. $\times 125$.

69 Taken from a section 530 microns behind figure 68. It passes through that part of the brain in which there are accumulated a great number of giant cells (*M'.C'*.) in the region of the roof plate. As in figure 68, there is an isolated cavity near the dorsal surface, which was probably a portion of the large embryonic central canal, but which in the adult is separated from the central canal and from the more anterior isolated dorsal cavity by ependyma. It seems best to the writer to regard this and the preceding dorsal cavity as vestigial structures. $\times 125$.

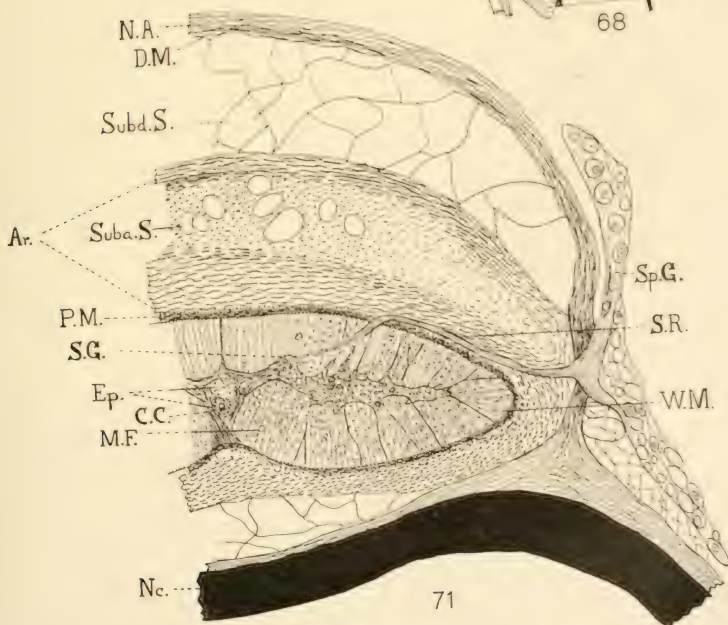
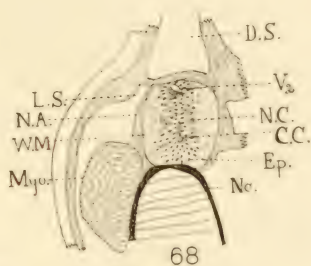
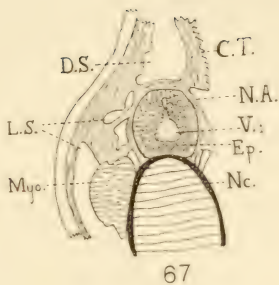
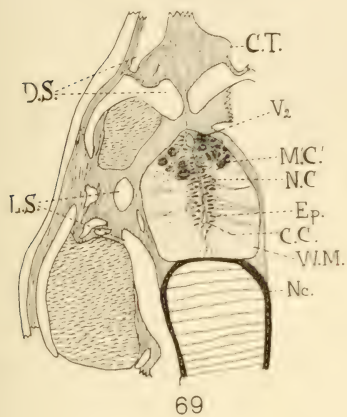
70 (See next plate.) Transverse section through the anterior spinal cord from the same series as the three previous figures. Observe that the *Amphioxus* spinal cord is not depressed as is the *Cyclostome* spinal cord, but is indented ventrally by the notochord. The central canal, which in some places exists as a dorso-ventral cleft, is almost obliterated here by the ingrowth of ependymal tissue. $\times 125$.

71 Cephalic transverse section through a portion of the spinal cord, meningeal membranes, neural arch, notochord, spinal ganglion, and sensory root of an adult *Polistotrema*. Observe the depression of the spinal cord, its ventral indentation, the ventral or permanent central canal (*C.C.*), which contains Reissner's fiber, and immediately above, the dorsal portion of the embryonic central canal, which is here more or less filled with ependymal cells and their processes. It will be seen that the gray matter is as much flattened out as is the cord itself, and the ventral horn and motor cells are crowded laterad, while the dorsal horn, *substantia gelatinosa* (*S.G.*), is apparently median and dorsal. Within the neural arch there is abundant room for a spherical spinal cord. The cord is held in place by the usual meningeal membranes. $\times 70$.

ABBREVIATIONS

<i>Ar.</i> , Arachnoidea	<i>Nc.</i> , notochord
<i>C.C.</i> , central canal	<i>P.M.</i> , pia mater or meningeal membrane of the younger stages
<i>C.T.</i> , white fibrous connective tissue	<i>S.G.</i> , <i>substantia gelatinosa</i>
<i>D.M.</i> , dura mater	<i>Sp.G.</i> , spinal ganglion
<i>D.S.</i> , dorsal veno-lymphatic sinus	<i>S.R.</i> , sensory or dorsal spinal nerve root
<i>Ep.</i> , ependyma	<i>Suba.S.</i> , subarachnoid cavities
<i>L.S.</i> , lateral veno-lymphatic sinus or anlage of the same	<i>Subd.S.</i> , subdural spaces
<i>M'.C'</i> ., Müllerian or giant cells	<i>W.M.</i> , white matter
<i>M.F.</i> , Müllerian or giant fiber	<i>V.1.</i> , anterior ventricle <i>Amphioxus</i>
<i>Myo.</i> , myotomes	<i>V.2.</i> , vestiges of the embryonic central canal in <i>Amphioxus</i>
<i>N.A.</i> , membranous neural arch	
<i>N.C.</i> , nerve cell	

SPINAL CORD AND MEDULLA OF CYCLOSTOMES
WILLIAM F. ALLEN



EXPLANATION OF FIGURES

72 to 80 represent a number of transverse sections through the medulla of shark, amphibian, and pig embryos for the purpose of demonstrating various stages of roof plate expansion.

72 Rather oblique transverse section through the medulla of a 19 mm. *Squalus* embryo in the region of the VIII ganglion (from series No. 2 of Professor Seammon's collection). Note the well-formed fourth ventricle, and the broadly expanded and very much stretched roof plate. Its collapsed appearance in this section is doubtless due to fixation or preparation. On account of a great proliferation of cells and nerve fibers the lateral plates have fused ventrally, as in *Petromyzon*, obliterating that part of the embryonic central canal. Attention should be called to the fact that the medulla roof plate in sharks begins to expand much earlier than it does in *Petromyzon*. This figure shows a well-expanded roof plate, while the cells in the mantle layer are no more differentiated and there are no more nerve fibers in the marginal layer than appear in a 12 day *Petromyzon*-embryo (fig. 40), where there is no fourth ventricle and no expansion of the roof plate. $\times 70$.

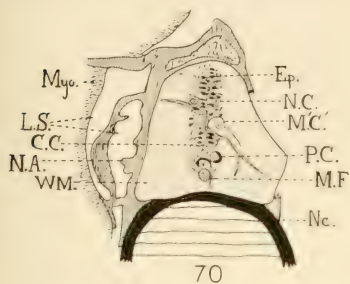
73 Transverse section through the medulla of a 15 mm. *Necturus* taken through the VIII ganglion. Observe the wide fourth ventricle and the broadly expanded and greatly stretched roof plate, and the coagulated appearance of the cerebro-spinal fluid in the ventricle. Like *Squalus*, the fourth ventricle begins relatively much earlier than in *Petromyzon*. It should be noted that no blood vessels have reached the level of the roof plate or entered the medulla; hence the coagulum in the ventricle must be largely a product of secretion. $\times 39$.

74 From a transverse section of a very young *Amblystoma* embryo in the region of the auditory vesicle (Professor Johnston's series No. 50). Here there has occurred a dorsal and a smaller ventral excavation of the cleft-like central canal. It will be seen that the larger dorsal cavity, the beginning of the fourth ventricle, possesses no thinner roof plate than does the spinal cord (fig. 83). Also in this section (fig. 74) the roof and floor plates are about equally thick. What has taken place dorsally and ventrally throughout the embryonic central canal has been a migration of the cells outward. The fact that in this section of the medulla the roof plate is no thinner than in the section of the spinal cord (fig. 83), taking note that the spinal and cranial ganglia are well-formed, is evidence against the hypothesis, that the greater migration of the neural crest cells of the medulla was the prime cause of the thinning out of the roof plate of the rhombic brain. $\times 70$.

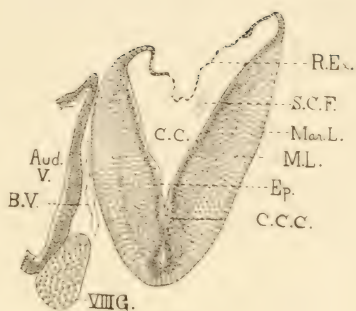
(Continued on page 74)

ABBREVIATIONS

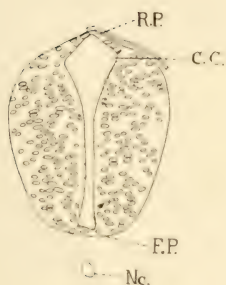
<i>Aud.V.</i> , auditory vesicle or otocyst	<i>M.L.</i> , mantle layer
<i>B.V.</i> , blood vessel	<i>Myo.</i> , myotomes
<i>C.C.</i> , central canal or cast of the same	<i>N.A.</i> , membranous neural arch
<i>C.C.C.</i> , central canal closure, caused by fusion of lateral plates	<i>N.C.</i> , nerve cell
<i>Ep.</i> , ependyma	<i>Nc.</i> , notochord
<i>Ep.N.</i> , layer of ependymal nuclei	<i>P.C.</i> , pigmented or eye cells of <i>Amphioxus</i>
<i>F.P.</i> , floor plate of the central nervous system	<i>R.Ex.</i> , roof plate expansion
<i>G.C.</i> , germinal cell	<i>R.P.</i> , roof plate of the central nervous system
<i>L.S.</i> , lateral veno-lymphatic sinus or anlage of the same	<i>S.C.F.</i> , cerebro-spinal fluid
<i>Mar.L.</i> , marginal layer	<i>V.G.</i> , Gasserian or semilunar ganglion
<i>M'.C'</i> , Müllerian or giant cells	<i>VIII.G.</i> , Auditory ganglion
<i>M.F.</i> , Müllerian or giant fiber	<i>W.M.</i> , white matter
	<i>4 V.</i> , fourth ventricle



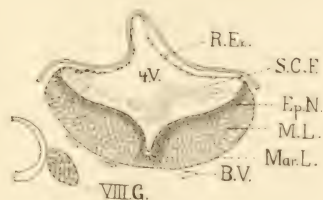
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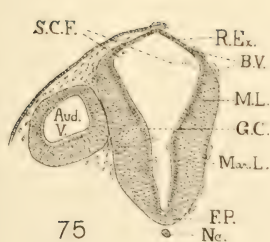
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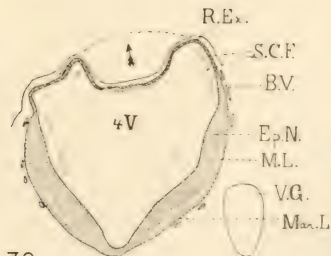
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75 to 80 represent five transverse sections through the developing fourth ventricle and roof plate expansion in pig embryos from 5 mm. up to 14 mm. With the exception of figure 75, which is from my collection, the remaining figures are from frontal series belonging to the Institute of Anatomy. That there is a direct relationship between the amount of visible coagulum in the form of a fibrillar feltwork and the expansion of the roof plate is evidenced by the fact that this coagulum does not appear in the early embryos before the roof plate has assumed the appearance of an organ capable of the production of cerebro-spinal fluid (as indicated by vascular supply and granular appearance of the cells). It may be inferred that the earliest non-coagulable cerebro-spinal fluid in the earliest stages is an embryonic fluid which differs in no way from the ordinary intercellular juices, but that the appearance of coagulum at the time when the roof plate has attained the appearance of a functional chorioid plexus is indicative of a chemical change in the fluid, which if a product of secretion is capable of producing a marked increase of internal pressure in the cerebro-spinal fluid and consequent expansion of the roof plate.

75 From a transverse section through the medulla of a 5 mm. (or less) pig embryo, in the region of the auditory vesicle. It will be seen that the peripheral branches of the intersegmental blood vessels have about reached the roof plate, but no blood vessels have entered the medulla. The protoplasm of the inner margin of the ependymal cells is sufficiently granular to suggest a secretory function. The small amount of coagulum in ventricle is probably the result of secretion, but the cerebro-spinal fluid has probably not exerted much internal pressure. $\times 70$.

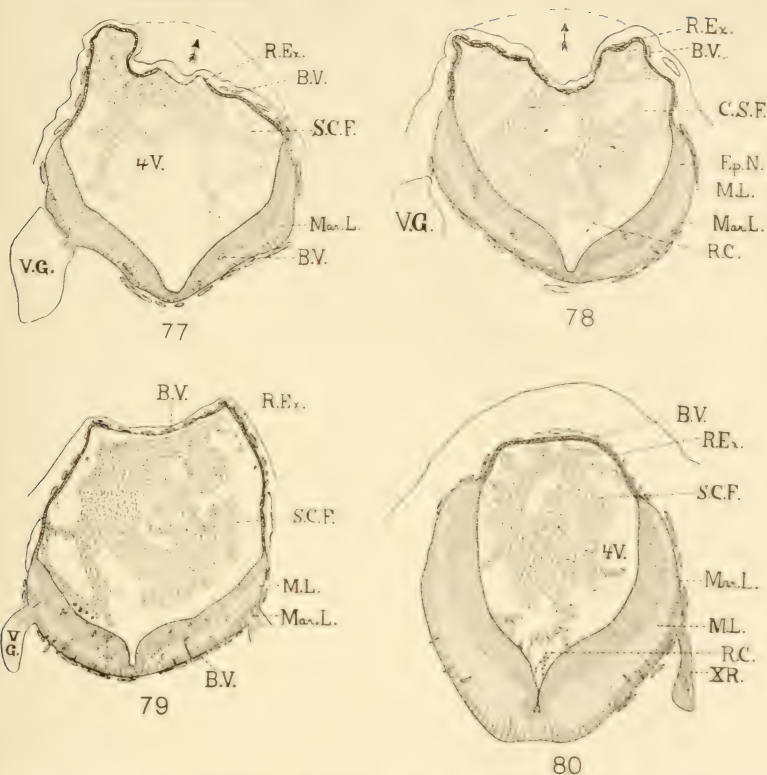
76 Transverse section of a 6 mm. pig medulla through the widest portion of the fourth ventricle, namely, at the level of the V ganglion. This is the only portion of the roof plate to have undergone any stretching of its cells, and this is confined solely to the most centrally located cells. This section shows a considerable increase in the size of the fourth ventricle and expansion of the roof plate, together with some increase in the amount of coagulable cerebro-spinal fluid (*S.C.F.*) and an increase in the number of blood vessels above the roof plate; but no blood vessels have entered the substance of the medulla. At this stage the pontine flexure could not have been a factor in producing the roof expansion. The collapsed appearance of the roof plate at its center is not natural, but rather a result of the preparation of the material. $\times 39$.

PLATE 15

EXPLANATION OF FIGURES

77 Taken from a transverse section through the V ganglion of a 7 mm. pig embryo. Note the increase in the number of blood vessels above the roof plate, which together with the increase in the coagulable cerebro-spinal fluid suggests a functional chorioid plexus. The pontine flexure in this stage is too slight to have any effect on the expansion of the roof plate. It should also be recorded that a few blood vessels have entered the outer surface of the medulla. As in the previous series the sections have suffered a collapse of the roof plate from fixation or later preparation of the material. $\times 39$.

78 From a transverse section of a 10 mm. pig embryo through the region of the V ganglion. The increased vascularity of the mesenchyme above the roof plate together with the enormous amount of coagulated cerebro-spinal fluid (*S.C.F.*) in the ventricle are evidences of the factors which have produced the increased expansion noticed in the roof of the ventricle. Also at this stage the pontine flexure has increased to such an extent that its action on a fourth ventricle full of cerebro-spinal fluid, itself under a moderate pressure, would produce a further expansion of the roof plate. As in the preceding sections the roof plate has suffered a collapse in the preparation of the material. $\times 39$.



79 and 80 Two transverse sections through the anterior and posterior ends of the fourth ventricle from a 14 mm. frontal series of a pig. A more advanced stage in the development of the chorioid plexus together with a more pronounced pontine flexure has produced a much larger fourth ventricle and expanded roof plate than is shown in the previous series (fig. 78). The thickening of the lateral walls of the medulla is taking place as it did in *Petromyzon* and *Squalus*, but the greater expansion of the ventricle in the pig (fig. 79) has prevented the walls from fusing ventrally. Nevertheless, the thickening of the ventral portion of the lateral plates would increase the pressure of the cerebro-spinal fluid. $\times 25$ and 39.

ABBREVIATIONS

B.V., blood vessel
C.S.F., cerebro-spinal fluid
Ep.N., layer of ependymal nuclei
Mar.L., marginal layer
M.L., mantle layer
R.C., embryonic red corpuscle

R.Ex., roof plate expansion
S.C.F., cerebro-spinal fluid
V.G., Gasserian or semilunar ganglion
4 V., fourth ventricle
X.R., vagus root

PLATE 16

EXPLANATION OF FIGURES

S1 to S7 Represent true transverse sections through what has been termed in the text, the typical embryonic spinal cord, from a number of different vertebrates, all of which have developed a tubular nervous system after the neural fold method. They were drawn with the aid of an Edinger-Leitz drawing apparatus and reduced one half in reproduction.

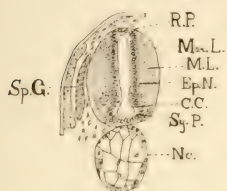
S1 From a transverse section through the anterior portion of the spinal cord of a 10 mm. *Squalus* embryo (Professor Scammon's series No. 16). This so-called typical embryonic spinal cord is decidedly compressed. An earlier stage possessed an elliptical cord with its greatest diameter from side to side. The floor plate is slightly thicker than the roof plate. Each contains a single layer of nuclei. The ventral portion of the cleft-like central canal is expanded into a cavity, which persists as the permanent central canal. A well-formed spinal ganglion is seen to the left. $\times 125$.

S2 Transverse section of the spinal cord of a 19 mm. *Squalus* embryo (from Professor Scammon's series No. 2). Note that the dorsal closure of the lateral plates, due to fiber and cell proliferation, is the same as was figured for *Petromyzon*. They meet in a seam, leaving dorsal and ventral cavities, of which only the ventral one persists. As in *Cyclostomes* this method of closure would tend to throw a large part of the embryonic cerebro-spinal fluid into the brain cavities. $\times 70$.

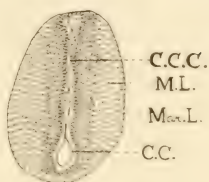
S3 and S4 Transverse section through the anterior portion of the spinal cord of an *Amblystoma* and a turtle embryo. The former (taken from Professor Johnston's series No. 50) is a rather early representative of the so-called typical embryonic stage; while the latter is a rather late representative of this stage. Both cords may be said to be compressed (elliptical, having its greatest diameter dorso-ventral), but only slightly so, when compared with birds and mammals (figs. S5 and S6). As a result, granting an equal proliferation of fibers and cells in the lateral plates, it would be expected that the adult cord in *Amblystoma* and the turtle would be more depressed, which is found to be the case. $\times 70$ and 125 .

S5 and S6 From anterior transverse sections of the spinal cord of a 93 hour chick and a 5 mm. pig. Both are good illustrations of the so-called typical embryonic stage, the pig being in a slightly more embryonic state. In these we have the most compressed of all embryonic cords examined, while the adults cord are nearly cylindrical. $\times 125$.

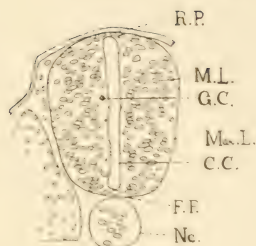
S7 Transverse section through the caudal end of the same spinal cord shown in figure S6. Observe spherical appearance which is indicative of an earlier phase in its development. $\times 125$.



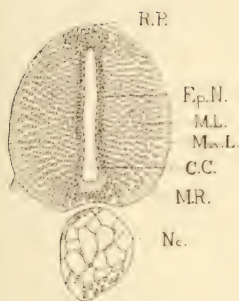
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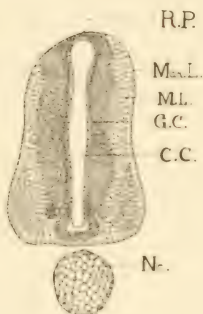
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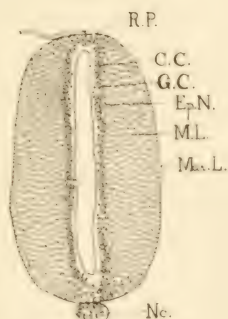
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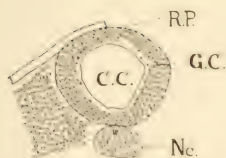
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ABBREVIATIONS

- | | |
|---|--|
| C.C., central canal | M.L., mantle layer |
| C.C.C., central canal closure, caused by fusion of lateral plates | M.R., motor or ventral spinal nerve root |
| Ep.N., layer of ependymal nuclei | Ne., notochord |
| F.P., floor plate of the central nervous system | R.P., roof plate of the central nervous system |
| G.C., germinal cell | Sp.G., spinal ganglion |
| Max.L., marginal layer | Sy.P., syncyetium of protoplasm |

MORPHOLOGY OF THE ROOF PLATE OF THE FORE-BRAIN AND THE LATERAL CHOROID PLEXUSES IN THE HUMAN EMBRYO

PERCIVAL BAILEY

From the Anatomical Laboratory of the University of Chicago

THIRTY-ONE FIGURES

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INTRODUCTION

The researches of Minot, von Kupffer, Burekhardt, G. Elliot Smith, C. Judson Herrick, J. B. Johnston and others, have succeeded in homologizing with considerable certainty the structures in the roof of the prosencephalon of the lower vertebrates, and of some of the lower Mammalia. The results of these comparative studies Johnston extended to the human embryo and it seemed desirable to examine some other human embryos of different ages in the hope that additional light might be shed on the method of development of these structures.

HISTORY

Concerning the structures with which this study is concerned, the writings of such authors as Faivre ('54), Lusehka ('85), and Haeckel ('60), previous to the work of Wilhelm His, contain very little of value.

Of the choroid plexus of the lateral ventricle, His writes ('04):

Sein dem Thalamus angehefteter Randstreifen bleibt endymal und in ihm bildet sich die Fissura chorioidea, von der aus die Epithelfaltungen des Corpus chorioideum in den Seitenventrikel sich einstülpen.

Minot ('01) considers the lateral plexus to be developed from the velum transversum.

In both birds and mammals the lateral portions of the velum, i.e., the choroid plexus of the lateral ventricle is highly developed. It thus appears that as we ascend the vertebrate series there is first a broadening of the velum, and an increase in its lateral development, then occurs a further reduction and flattening out of the velum, and a much greater growth of the lateral plexus.

G. Elliot Smith ('03) attempted another explanation for the formation of the lateral plexus.

Now, although in the whole of its extent the epithelial layer of the choroid plexus presents uniform features, it is difficult to admit a common origin for the whole structure; with regard to that part of the plexus which is found in the region of the foramen of Monro, there can be little doubt of its origin from the primitive roof of the fore-brain. . . . But the case is very different with that portion of the plexus which is not directly connected with the roof of the forebrain, but is attached to the stria terminalis. There is no evidence to show that this portion is derived from the roof, and all the facts of development point to the conclusion that its proximal attachment to the optic thalamus is a primitive and not a secondarily acquired relation. Such being the case, the caudal extension of the epithelial choroidal fold in the mammalian hemisphere would appear to be derived from a stretching of the attachment of the labium caudale of the cerebral hemisphere to the optic thalamus. As a result of this, the connecting band becomes reduced to an epithelial lamina, which becomes invaginated and folded by an extension backward of the choroidal folding which begins farther forward in the region of the foramen of Monro.

Johnston ('09) after showing that the velum transversum is continued down the side-wall of the prosencephalon as the diencephalic groove, returns to a modification of Minot's original idea.

In the angle between the [cerebral] vesicle and the diencephalon appears the choroid plexus pushing into the lateral ventricle. It appears as a folding of the anterior limb or wall of the velum transversum and its lateral prolongation [di-telencephalic groove].

Concerning the mesodermal portion of the lateral plexus, Meek ('07) makes the following statement.

The choroid plexuses of the lateral ventricles are due to an ingrowth of the pia mater pushing the mesial wall of the hemispheres into the ventricles.

This is the current notion.

The arachnoid is not supposed to be present, although the plexus is but a fringe of the velum interpositum, into the structure of which the arachnoid does enter. The neural wall is, of course, preserved, but consists only of a simple epithelium. The plexuses are then thin laminae covered with an epithelium, beneath which is a connective tissue stroma containing an extraordinarily rich network of blood-vessels.

Findlay's ('99) idea of a single membrane, the pia-arachnoid, removes the difficulty concerning the involvement of the arachnoid.

Finally, Hochstetter ('13) has written a purely descriptive account of the development of the lateral choroid plexus with no attempt to analyze its parts. He summarizes his work as follows:

Fassen wir das bisher Mitgeteilte zusammen, so können wir sagen, dass sich die Plexus chorioidei der Seitenventrikel ungefähr in derselben Richtung entwickeln wie die Hemisphärenblasen selbst. Zuerst angelegt, wenn auch nicht gleich als Anlage der Plexus chorioidei kenntlich, ist ihr vorderster, im Bereiche der Decke des Cavum Monroi befindlicher Abschnitt. Er entsteht hier, wie wir gesehen haben, aus den die Sulci hemisphaerici [di-telencephalic grooves] bildenden Hirnwandfalten, sowie aus dem diese beiden, in der Fortsetzung des Zwischenhirndaches verbindenden, vorerst kielförmig vorspringenden Wandteile des Endhirns. Ein zweiter Abschnitt erscheint wesentlich später in Form einer jederseits zunächst einfachen gegen den Hohlraum der Seitenkammer zu vorspringenden Falte, der als Area chorioidea bezeichneten Wandplatte der Hemisphäre. Diese Falte geht vorn in die Wandfalte des Sulcus hemisphaericus über, während sie sich nach rückwärts etwas von ihr entfernt (fig. 4), noch weiter nach rückwärts aber bald verstreicht. So erscheint bei dem ältesten von den drei

bisher besprochenen Embryonen (H. Sch. 2) der hinterste dem Zwischenhirn anliegende Abschnitt der Area chorioidea nach vollkommen glatt und ungefaltet.

All of his figures, with the exception of figure 6, appear to be through the diencephalon, back of the velum transversum.

The development of the paraphysis in the human embryo has never been followed. In fact, its identification is very much in doubt. Francotte ('94) claims to have found it in an embryo of twelve weeks. It is said to be an organ characteristic of all vertebrates, but becomes very rudimentary in birds and mammals. Its development has been followed by Dexter ('02) in the common fowl, and it has been described by Selenka ('91) in the opossum. A good review of the literature is given by Warren ('05).

Of the human embryo, Streeter in Keibel and Mall's Human Embryology says,

Orally this choroid roof [of the third ventricle] is continued into the telencephalon where it forms a pointed pouch overlapping the lamina terminalis and the contained commissures. . . . The anterior choroidal pouch has been homologized with the paraphysis of the lower vertebrates.

It should be borne in mind that in all vertebrates, the paraphysis, if present, arises from the roof of the telencephalon just cephalad to the velum transversum. In view of this fact, the structure labelled paraphysis by Goldstein ('03) is obviously not so, since it lies behind a structure called the velum transversum and at the posterior end of the diencephalic roof.

The epiphysis is a constant organ in the vertebrate series (except in the alligator) but probably concerning no other organ has there been so much confusion and misinterpretation. For a review of the literature, reference may be had to Gaupp ('98). The development of the epiphysis has not been followed completely in the human embryo, and so far as I know, an indication of the division into the two parts, epiphyseal stalk and pineal vesicle, which seems to be so characteristic of many vertebrates, has never been recorded.

With the recognition of the velum transversum in Cyclostomes by Sterzi ('07), the velum has been established as a constant

morphological feature in the roof of the prosencephalon of vertebrates.

The tela chorioidea diencephali has been the subject of an extensive anatomical, embryological and comparative study by Lachi ('88). It presents few features of interest. A number of names have been applied to parts of it (Zirbelpolster, dorsalsac, post-paraphysis, post-velar arch, etc.), but it has generally been recognized as extending from the superior commissure to the velum transversum.

With regard to the roof of the telencephalon, however, there is no such unanimity of opinion. I shall not attempt to review the observations on the lower vertebrates. Johnston ('13) reviews the literature fully and on the basis of this and his own extensive observations presents the following scheme as covering all the forms of the roof of the telencephalon, beginning with the preoptic recess in which the sulcus limitans ends:

- Lamina terminalis (containing the anterior commissure)
- Recessus neuroporicus
- Lamina supraneuroporica (containing the pallial commissures)
- Recessus superior
- Tela chorioidea telencephali
- Paraphysis
- Velum transversum (anterior leaf)

Instead of tela chorioidea telencephali, the more definite term, tela chorioidea telencephali medii, will be used in this paper.

This plan may be completed for the diencephalon as follows:

- Velum transversum (posterior leaf)
- Tela chorioidea diencephali
- Commissura superior
- Epiphysis

The posterior commissure belongs to the mesencephalon. The neuropore itself has never been followed through the successive stages of its development in the human embryo.

The evidence for transferring the above scheme to the human embryo is not wholly conclusive, and the present work was undertaken primarily to determine in how far the morphology in this region in certain human embryos was compatible with the above scheme.

I wish here to express my indebtedness to Dr. C. J. Herriek, whose broad knowledge and mature judgment has been of invaluable assistance, and especially to Dr. Geo. W. Bartlmez, at whose suggestion the work was undertaken and without whose kindly interest the work would have been impossible. Thanks are also due to Mr. A. B. Streedain for the care he has taken with the illustrative work.

MATERIAL AND METHODS

The material upon which this study was chiefly based consists of three very well preserved human embryos, cut in transverse series, stained in bulk with borax carmine, and counterstained on the slide with orange G. Wax-plate reconstructions were made, the plates being stacked from a side view of the embryo drawn from a photograph, taken after fixation. The shrinkage after embedding was calculated and the outline reduced accordingly. Although the primary object of this study is the morphology and relations of the roof plate, in two cases the entire forebrain has been modeled. This was done because the embryos happened to fall in at opportune intervals between His' embryo CR (13.6 mm.) and the embryo of 50 mm. also modeled by him, and also in order that the relations of the choroid plexuses might be seen more clearly.

Embryo H 173 was obtained from an aborted ovum of 42 x 32 x 19 mm., presented by Dr. N. R. Engels of Chicago. The only available history was that the patient had missed two menses. The intact ovum was placed in physiological salt solution and kept at about 0°C. for 11 hours. It was then opened and fixed in formalin-Zenker for 24 hours, stained in bulk with borax carmine, imbedded by the celloidin-paraffin method and cut 10 μ thick in a plane parallel to the hindbrain. The embryo measured 19.1 mm., crown-rump length after fixation. The sections were counterstained on the slides with orange G. The total shrinkage was about 20 per cent. There are frequent mitoses in embryo and chorion. The brain was modeled at a magnification of 50 diameters with the aid of the Edinger pro-

jection apparatus. One millimeter plates were used and every other section drawn except in the region of the foramen interventriculare, where half millimeter plates were used and every section drawn. The epiphyseal region was modeled at a magnification of 100 diameters. Millimeter plates were used and every section was drawn.

Embryo H 91 was obtained from an aborted ovum 50 x 34 x 30 mm. presented by Dr. G. C. Dittmann of Chicago, whose data indicate a clinical pregnancy of 60 days. The ovum was left unopened in physiological salt solution for 10 hours, then opened and fixed in an 8 per cent solution of formaldehyde, neutralized with magnesium carbonate. It measured 27.8 mm. crown-rump length after fixation in formalin and showed a shrinkage of 13.6 per cent after imbedding in paraffin. It was stained in bulk in borax carmine and on the slide with orange G. It was cut in 20μ sections, and modeled at a magnification of 40 diameters. Millimeter plates were used and every fifth section was omitted.

Embryo H 41 was obtained from an ovum of 71 x 39 x 32 mm., presented by Dr. L. A. Beaton of Chicago. The chorion was opened and the entire ovum fixed in formalin. The crown-rump measurement of the embryo after fixation was 32.1 mm. and it showed a shrinkage of 10 per cent after imbedding in paraffin. The staining was the same as that of H 91. This embryo was sectioned 20μ in paraffin, and modeled at a magnification of 25 diameters. Millimeter plates were used and every other section was drawn. The region around the foramen of Monro was modeled at a magnification of 100 diameters; 2 mm. plates were used and every section was drawn.

Two points in the technical procedure are to be emphasized because they are in large measure responsible for the exceptionally good preservation of the form relations of the delicate roof plate of the brain. Both of the older embryos (H 91 and H 41) had the cranial cavity opened by an incision in the line of the sagittal suture. Distortions due to unequal shrinkage of the brain and overlying structures were thereby in great measure avoided. All three were passed from 95 per cent alcohol to

ether-alcohol, then through 0.5, 1, 2 and 3 per cent celloidin, hardened in chloroform-alcohol, cleared in benzol and imbedded in paraffin under the air pump.

The plane of section in each case is shown in figures 29; 30 and 31.

Several other human embryos were studied, the most helpful being embryo H 44 of the Chicago collection, which measured 60.4 mm. after fixation in formalin. A transverse series from a 25 mm. pig in the collection of Dr. F. R. Lillie was also used.

DESCRIPTION

1. The 19 mm. embryo (H. 173)

The recessus preopticus is well marked (fig. 18, *r.pre.*). The roof plate stretches dorsad from this recess as a thickened lamina (fig. 18, *l.t.*) to about the level of the sulcus separating the medial and intermediate roots of the corpus striatum. Above this point the roof plate narrows and extends cephalad and dorsad (fig. 18, *l.s.?*), forms a broad arch (fig. 18, *r.s.*), and then passes caudad and dorsad (fig. 18, *t.c.t.m.*) as a still thinner membrane toward the velum transversum. Just in front of the velum transversum, the roof plate forms a small narrow arch (fig. 18, *p.a.* and fig. 2, *p.a.*), from the sides of which arise the lateral choroid plexuses.

(Throughout these descriptions, narrow and wide are used of dimensions tangential to the ventricular surface, and thick and thin of dimensions perpendicular to the ventricular surface. For example, in figure 12, the tela chorioidea diencephali is thin and wide.)

The velum transversum is well marked (fig. 18, *v.t.*), indicating the boundary in the roof plate between the diencephalon and telencephalon.

The roof of the diencephalon (fig. 18, *t.c.d.*) is still narrow throughout most of its extent. It is also relatively thick, with several rows of nuclei in cross-section. It is narrowest at its posterior end and remains narrow almost to the anterior end of the thalamus, where it suddenly widens (fig. 1, *t.c.d.*). The

entire structure, when viewed from above, is somewhat trumpet-shaped, with the bell at the anterior end. There is no indication of plexus formation. The entire roof plate of the diencephalon is perfectly smooth. The commissura superior is clearly indicated (fig. 18, *com.s.*).

The epiphyseal evagination is a hollow outgrowth (figs: 18 and 3, *e.e.*). The top of the evagination is cupped and in this cup lies a ball of cells (figs. 3, 10 and 18, *e.v.*). This ball of cells has an irregular lumen in its center (fig. 10, *e.v.*). The cells of the ball stain more lightly than the cells of the cup, and the line of separation is fairly distinct. It will be seen that this ball of cells, while not in actual contact with the epidermis, approaches it very closely (fig. 10, *e.v.*). The epiphyseal evagination lies some distance cephalad of the commissura posterior (fig. 18, *c.p.*).

The extent and morphology of the lateral choroid plexus is shown in figure 19. It is clearly divisible into two parts, an anterior part (figs. 2 and 19, *p.c.r.l., p.a.*) attached to the lateral margin of the paraphysal arch along its entire length, and by the taenia fornicis (fig. 2, *t.f.*) to the medial hemisphere wall immediately above and lateral to it; and a posterior part (figs. 1 and 19, *p.c.r.l., p.p.*) attached by the taenia chorioidea (fig. 1, *t.c.*) to the lateral thalamic wall and by the taenia fornicis (fig. 1, *t.f.*) to the medial hemisphere wall immediately below the hippocampus. The fissura chorioidea is very wide throughout most of the extent of the posterior part of the lateral choroid plexus.

If the angle between the taenia chorioidea and the thalamic wall be followed anteriorly, it is found to be continuous with the velum transversum; if the angle is followed posteriorly, it continues backward between the lateral thalamic wall and the medial hemisphere wall, turns downward between them and passes anteriorly and downward behind the optic nerve on the lateral wall of the hypothalamus (fig. 19, *d-t. gr.*). This groove is more clearly marked on the lateral wall of the 32 mm. embryo.

The ependymal portion of the plexus is still relatively thick (fig. 27). The mesenchymal tissue of the plexus is typical em-

bryonal connective tissue. The blood capillaries are particularly numerous near the ependyma.

If one examines now the ventricular surface, the corpus striatum (fig. 19, *c.s.*) appears at the posterior point of attachment of the lateral plexus as a single ridge in the floor of the lateral ventricle. As it is followed anteriorly, this ridge is soon divided by a groove into two portions. The lateral portion is

REFERENCE LETTERS

- | | |
|---|---|
| <i>a.c.a.</i> , area chorioidea anterior. | <i>n.post.</i> , recessus postopticus |
| <i>a.c.p.</i> , area chorioidea posterior. | <i>o.n.</i> , optic nerve |
| <i>a.p.</i> , anterior pouch (of the tela chorioidea diencephali) | <i>p.a.</i> , paraphysal arch |
| <i>aq.S.</i> , aqueduct of Sylvius | <i>p.c.v.l.</i> , plexus chorioideus ventriculi lateralis |
| <i>cb.</i> , cerebellum | <i>p.c.v.l.</i> , <i>p.a.</i> , plexus chorioideus ventriculi lateralis, pars anterior |
| <i>c.o.</i> , chiasma opticum | <i>p.c.v.l.</i> , <i>p.p.</i> , plexus chorioideus ventriculi lateralis, pars posterior |
| <i>com.s.</i> , commissura superior | <i>p.o.hy.</i> , pars optica hypothalami |
| <i>c.p.</i> , commissura posterior. | <i>r.m.</i> , recessus mamillaris |
| <i>c.s.</i> , corpus striatum | <i>r.n.</i> , recessus neuroporicus |
| <i>c.s.i.r.</i> , corpus striatum, intermediate root | <i>r.post.</i> , recessus postopticus |
| <i>c.s.l.r.</i> , corpus striatum, lateral root | <i>r.pre.</i> , recessus preopticus |
| <i>c.s.m.r.</i> , corpus striatum, medial root | <i>r.s.</i> , recessus superior |
| <i>d.p.</i> , deep pit (in telencephalic roof plate) | <i>s.a.</i> , striatal area |
| <i>d-t.gr.</i> , di-telencephalic groove | <i>s.l.</i> , sulcus limitans |
| <i>e.e.</i> , epiphyseal evagination | <i>s.m.</i> , stria medullaris |
| <i>ep.</i> , epidermis | <i>s.M.</i> , sulcus Monroi |
| <i>e.r.</i> , epiphyseal ridge | <i>s-t.</i> , subthalamus |
| <i>e.v.</i> , epiphyseal vesicle | <i>t.c.</i> , taenia chorioidea |
| <i>f.a.</i> , fissura arcuata | <i>t.c.d.</i> , tela chorioidea diencephali |
| <i>f.c.</i> , fissura chorioidea | <i>t.c.t.m.</i> , tela chorioidea telencephali medii |
| <i>f.int.</i> , foramen interventriculare | <i>t.f.</i> , taenia fornicis |
| <i>f.r.</i> , fasciculus retroflexus (Meynerti) | <i>tg.</i> , tegmentum |
| <i>h.a.</i> , and <i>hip.</i> , hippocampal area | <i>th.</i> , thalamus |
| <i>hem.</i> , hemisphere | <i>th.1.</i> , <i>th.2.</i> , parts of thalamus |
| <i>h.s-t.r.</i> , habenulo-subthalamic ridge | <i>th.</i> , <i>c.s.</i> , thalamus, ependymal surface |
| <i>hy.</i> , hypothalamus | <i>th.</i> , <i>p.s.</i> , thalamus, pial surface |
| <i>inf.</i> , infundibulum | <i>t.i-c.</i> , taenia infrachorioidea |
| <i>l.s.</i> , lamina supranuroporica | <i>t.r-p.</i> , telencephalic roof plate |
| <i>l.t.</i> , lamina terminalis | <i>t.s-c.</i> , taenia suprachorioidea |
| <i>mt.</i> , metathalamus | <i>t.t.</i> , taenia thalami |
| <i>n.h.</i> , nucleus habenulae | <i>v.t.</i> , velum transversum |
| <i>n-p.a.</i> , neopallial area | |

at first in the floor of the lateral ventricle but anteriorly comes to lie in the lateral wall (figs. 2 and 23, *c.s.l.r.*). The medial portion

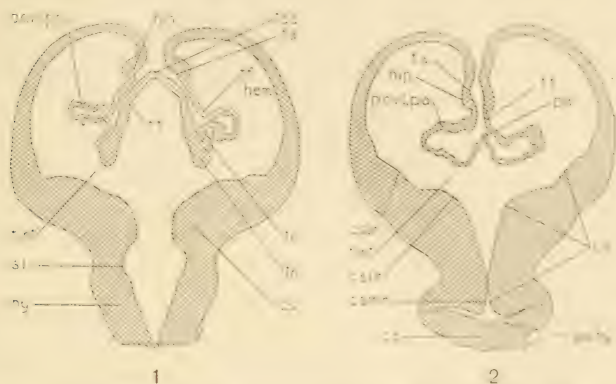


Fig. 1 Section through the diencephalon of the 19 mm. embryo, H 173. $\times 13\frac{1}{2}$. Slide 21, Sect. 11. Compare the photograph of this section, figure 27.

Fig. 2 Section through paraphysis and hypothalamus of the 19 mm. embryo, H 173. $\times 13\frac{1}{2}$. Slide 23, Sect. 13.

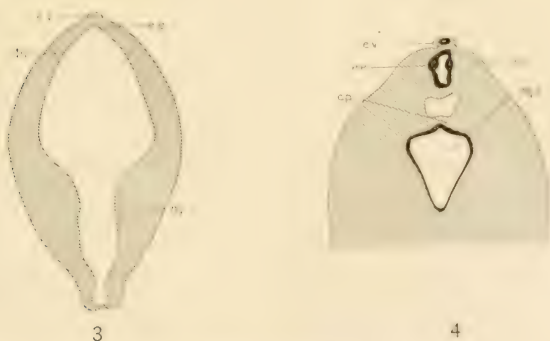


Fig. 3 Section through epiphysis of the 19 mm. embryo, H 173. $\times 15$. Slide 12, Sect. 4.

Fig. 4 Section through epiphysis of the 28 mm. embryo, H 91. $\times 10$. Slide 9, Sect. 14S. Ependyma solid black.

continues to the foramen interventriculare where it divides into two parts, the lateral part passing forward in the floor of the lateral ventricle (the intermediate root of the corpus striatum, figs. 2 and 23, *c.s.i.r.*), and the medial part passing through the foramen interventriculare, forming its floor, and extending in the lateral wall of the third ventricle behind the lamina terminalis as far as the recessus preopticus (fig. 18, *c.s.m.r.*). It is very likely that this elevation which I have just described as the medial root of the corpus striatum contains in its lower end other things besides striatal tissue. I shall continue to describe the entire elevation as the medial root of the corpus striatum, following the usage of His, and shall not enter into a discussion of its internal structure.

Above the taenia fornicis on the ventricular surface of the medial hemisphere wall may be seen an elevation (fig. 19, *hip.*) extending from the posterior pole of the hemisphere just above the choroid plexus, and beyond it over the foramen interventriculare. This is the anlage of the hippocampus, at least in part. There is corresponding to it on the outer pial side of the hemisphere wall, a shallow groove (figs. 1 and 2, *f.a.*). This groove is not due to the folding in of a thin weak place in the wall, for the wall at this point is very definitely thicker (fig. 27).

Turning now to the wall of the third ventricle, we find just below the tela chorioidea diencephali a low ridge (fig. 18, *n.h.*) extending from the epiphyseal evagination almost to the velum transversum. This is the habenula. From its anterior end a sharp ridge runs backward and downward to the subthalamus (fig. 18, *h.s-t.r.*). Above this ridge, the wall is shrunk and thin up to the habenular thickening. Below the ridge, is an elevation (fig. 18, *th.1*) which extends upward and forward in front of the habenula toward the velum transversum.

The sulcus limitans is indicated on figure 18 by a dotted line running above the tegmentum and subthalamus, below the elevation last mentioned (fig. 18, *th.1*) and behind the corpus striatum to the preoptic recess. Just back of the corpus striatum and between it and the hypothalamus, the sulcus limitans runs into a very deep recess (fig. 2).

Below the sulcus limitans lie the subthalamus (fig. 18, *s-t.*) and hypothalamus (fig. 18, *hy.*). The elevation which marks this region is very long. The pars optica is only indistinctly marked off (fig. 18, *p.o.hy.*). At its posterior end, the elevation divides into two portions, one of which, subthalamus, continues upward into the tegmentum, the other, hypothalamus, backward into the mammillary recess.

The floor plate is thin and somewhat widened (fig. 3). The infundibulum (fig. 18, *inf.*) lies a considerable distance back of the optic chiasm. The postoptic recess is but poorly marked (fig. 18, *r. post.*).

2. The 28 mm. embryo (*H. 91*)

The entire telencephalon is not modeled in this embryo. The model was made primarily to show the lateral choroid plexus. Those portions not modeled differ in no essential respect from the corresponding portions of the 32 mm. embryo.

Immediately cephalad of the velum transversum (fig. 20, *v.t.*), which is clearly indicated, the roof plate forms a small arch (fig. 20, *p.a.*) to the sides of which are attached the lateral choroid plexuses. In front of the arch, the roof plate becomes very thin for a few sections (fig. 5, *t.c.t.m.*). Then as we pass cephalad of this thin lamina, we come to a region where the roof plate thickens in a peculiar manner (fig. 6, *lr-p.*).

The hemisphere wall on each side of the roof plate is thin and in it two distinct zones are discernable, a broader zone next the ependymal surface, where the nuclei are very numerous, mantle layer, and a narrower zone next the pial surface which is relatively free from nuclei, marginal layer. In the roof plate, however, these two zones are not discernable, the nuclei are equally numerous throughout from the pial to the ependymal surface, and at the ependymal surface are loosely arranged, so that the outline is indefinite and irregular. This is the characteristic arrangement, and such an arrangement I have found in this region in five human embryos of about this age in the collection of the Department of Anatomy and in a 25 mm. pig embryo in the

collection of Dr. F. R. Lillie. In the embryo under consideration toward the anterior end of this region (fig. 7, *tr-p.*) the morphology is somewhat different from that shown in figure 6. In the mid-line again, the nuclei are evenly distributed from ependymal to pial surface and are very numerous. Immediately on either side, however, the wall is greatly thickened in such manner that,

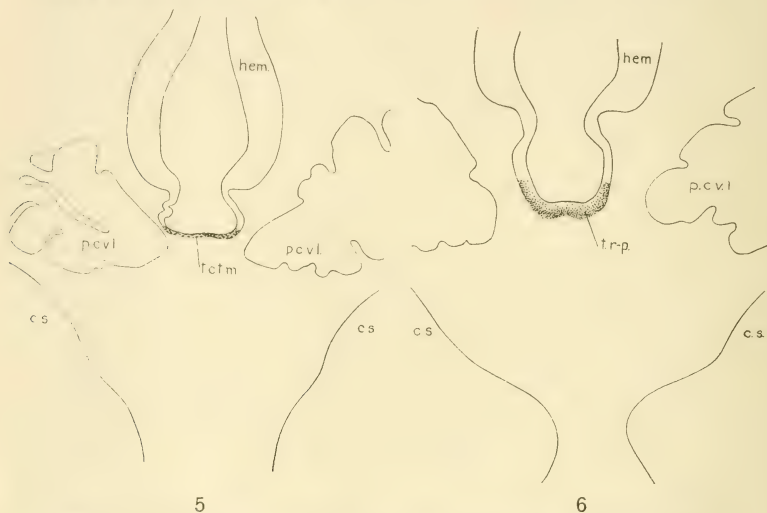


Fig. 5 Section through tela chorioidea telencephali medii of the 28 mm. embryo, H 91. $\times 50$. Slide 28, Sect. 362.

Fig. 6 Section through roof plate of telencephalon medium of the 28 mm. embryo, H 91. $\times 50$. Slide 29, Sect. 367.

although the pial surface still forms a regular curve, the ependymal surface shows a deep notch in the mid-line. The outline of the ependymal surface is again indefinite owing to the loosely arranged cells. The marginal layer approaches almost to the midline. At the extreme anterior end of this region the notch disappears; the ependymal outline becomes definite; and the roof plate thickens markedly in an undoubted lamina terminalis (fig. 20, *lt.*).

The tela chorioidea diencephali is very broad and very thin (fig. 11, *t.c.d.*). It does not exhibit any folding except at the extreme anterior end. Toward the posterior end of the tela, a narrow strip of the alar plate is curved lateralward, resembling the rhomboidal lip of the rhombencephalon, and which we may term the thalamic lip (fig. 9, *t.l.*).



Fig. 7 Section through the roof plate of telencephalon medium of the 28 mm. embryo, H 91. $\times 50$. Slide 29, Sect. 372.

Fig. 8 Section through the roof plate of telencephalon medium in the 32 mm. embryo, H 41. $\times 50$. Slide 32, Sect. 2.

The thalamic lip carries with it lateralward the taenia thalami (fig. 9, *t.l.*) and the roof plate. Toward the anterior end of the tela, the thalamic lip bends laterally more and more until its pial surface comes into contact with the pial surface of the thalamic wall (fig. 11, *t.l.*). The ependymal surface of the roof plate is hereby brought into contact with the ependymal surface of the thalamic lip (fig. 11). At the apex of the angle between the thalamic lip and the thalamic wall lies the stria medullaris (fig. 11, *s.m.*). The entire tela when viewed from above is wedge-

shaped, the anterior end being very broad. Heuser ('13) has noted a similar condition in the pig. The commissura superior is well marked (fig. 20, *com. s.*).

It will be seen that the roof of the epiphyseal evagination (fig. 20, *e.e.*) becomes epithelial for a short space in its uppermost portion. Just in front of this epithelial region there is a small aggregation of cells (fig. 4, *e.v.*) which recalls the ball of cells described in the epiphysis of the 19 mm. embryo. The nuclei

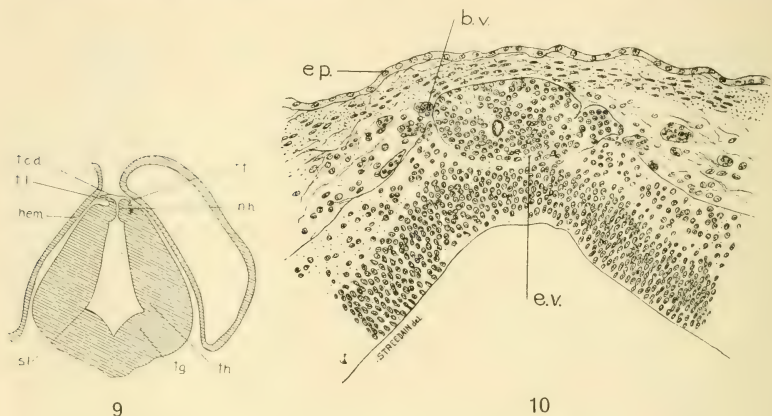


Fig. 9 Section through the diencephalon of the 28 mm. embryo, H 91. $\times 63$. Slide 15, Sect. 228.

Fig. 10 Section through the epiphysis of the 19 mm. embryo, H 173. $\times 150$. Slide 12, Sect. 4.

of these cells stain densely like the nuclei of the ependymal cells, and lie very close together. The cytoplasm is stained a deep yellow like the cytoplasm of the ependymal cells. They are surrounded by more lightly staining cells, and are nowhere in connection with the ependymal cells. The lateral wall of the epiphyseal evagination is very massive (fig. 4, *e.r.*). A ridge arises from the postero-superior portion of the lateral wall of the diencephalon and extends upward and backward to the epiphyseal evagination (fig. 21, *e.r.*).

The lateral choroid plexus (fig. 21, *p.c.v.l.*) is of considerable size, but does not nearly fill the ventricle (fig. 11, *p.c.v.l.*). In antero-posterior extent the plexus measures 1.55 mm., the ventricle measuring 2.97 mm. The anterior end of the plexus is much the larger and is less folded. Thompson ('09) has described a similar condition in the cat. The attachment of the plexus to the roof plate is now much narrowed, owing to the relatively small size of the paraphysal arch (fig. 20, *p.c.v.l.*, *p.a.*). The taenia fornicis is now closely approximated to the taenia chorioidea, the fissura chorioidea being reduced to a narrow

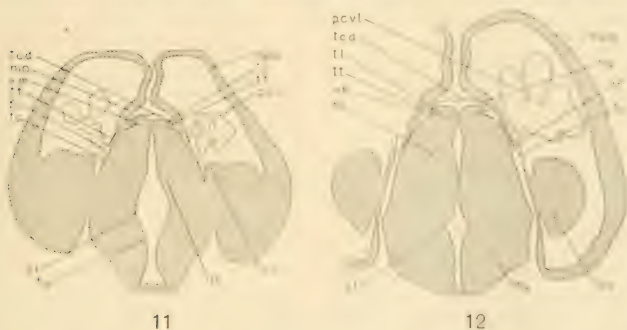


Fig. 11 Section through the diencephalon of the 28 mm. embryo, H 91. $\times 6\frac{2}{3}$. Slide 24, Sect. 318.

Fig. 12 Section through the diencephalon of the 32 mm. embryo, H 41. $\times 6\frac{2}{3}$. Slide 24, Sect. 2.

slit (fig. 11, *f.c.*). In its growth, the plexus has extended some 0.25 mm. anterior to its point of attachment to the mid-line of the telencephalon. The connective tissue resembles that of the 19 mm. embryo closely, except that the cells are farther apart, but the ependymal layer has become much thinner and consists clearly of a single layer of columnar cells, with the nuclei in the ends of the cells next the ventricular cavity (fig. 28). The ependyma of the posterior part of the plexus resembles more closely that of the 19 mm. embryo, there being several layers of irregularly arranged nuclei. The posterior part of the plexus is much attenuated and much more folded than the anterior end.

In the wall of the third ventricle, the habenula is well marked (fig. 20, *n.h.*) and from its posterior end a ridge extends downward to the tegmentum (fig. 20, *f.r.*). Just below the habenula, the wall of the diencephalon is much thicker than in the 19 mm. embryo, and extends anterior to the velum transversum to form the posterior wall of the foramen interventriculare.

3. The 32 mm. embryo (*H. 41*)

Just in front of the velum transversum, as in the 28 mm. embryo, the roof plate forms a low arch (figs. 22 and 25, *p.a.*) to the sides of which are attached the lateral choroid plexuses. Immediately anterior to this arch the roof plate becomes very thin (fig. 25, *t.c.t.m.*). Anterior to this thin lamina, the roof plate is thickened again in the manner noted in the case of the 28 mm. embryo (see fig. 6, *t.r-p.*). At the anterior end of this region (fig. 25, *l.s.?*), there is an indication of the median notch on the ependymal surface (fig. 8, *t.r-p.*). It is, however, not nearly so well marked as the similar notch in the 28 mm. embryo (fig. 7, *t.r-p.*). At the extreme anterior end of this region is a structure not found in any of the other embryos. A deep narrow pit extends into the roof plate from the pial surface, which causes the roof plate to project into the ventricle (figs. 13, 14, and 25, *d.p.*). This pit is found only in one section, with indications of it in the two adjacent sections. Immediately anterior to the pit, the roof plate thickens markedly and extends as a thickened lamina (fig. 22, *l.t.*) to the recessus preopticus. There is, however, a short distance anterior to the pit a shallow notch on the ventricular surface of the lamina terminalis (fig. 25, *r.n.?*).

The tela chorioidea diencephali shows only slight indications of longitudinal folding. The outwardly curved and very prominent thalamic lip (fig. 12, *t.l.*) is not in contact with the lateral wall of the thalamus. At the anterior end, the tela chorioidea diencephali is very broad, and a pouch (fig. 25, *a.p.*) arises which extends forward over the velum transversum. The whole tela resembles very closely the same structure in the 28 mm. embryo.

The commissura superior is very readily identified (fig. 22, *com.s.*).

The epiphyseal evagination (fig. 22, *e.e.*) is, in all essential respects, identical with that of the 28 mm. embryo.

The lateral choroid plexus (fig. 12, *p.c.v.l.*) also resembles that of the 28 mm. embryo. The posterior portion is more swollen, resembling more nearly the anterior part. The ependyma



Fig. 13 Section through the roof plate of the telencephalon medium of the 32 mm. embryo, H 41. $\times 50$. Slide 32, Sect. 4.

Fig. 14 Section through the roof plate of the telencephalon medium of the 32 mm. embryo, H 41. $\times 5$. Slide 32, Sect. 4.

is almost entirely a single layer of cells except at the extreme caudal end of the plexus. The plexus extends through 4.44 mm.; the ventricle through 7.2 mm. The plexus extends 1.6 mm. anterior to its most anterior point of attachment.

On the ventricular surface we find again that the corpus striatum exhibits three roots at its anterior end (fig. 24, *c.s.*). The most medial root, in the lateral wall of the third ventricle, is narrow, lies immediately back of the lamina terminalis, and

forms more the anterior boundary than the floor of the foramen interventriculare (fig. 22, *c.s.m.r.*). The intermediate root is also small, extending beyond the foramen interventriculare in the floor of the lateral ventricle. The intermediate root unites at its posterior end with the medial root, the two being separated from the lateral root by a groove. The lateral root is the largest, and lies more in the floor than in the lateral wall of the ventricle (fig. 24, *c.s.l.r.*). The two ridges, one formed by the lateral root and the other by the union of the medial and intermediate roots, extend backward some distance and then turn sharply downward as a single elevation (fig. 24).

The hippocampus (fig. 12, *hip.*) is a broad, slightly thickened portion of the medial hemisphere wall, just above the taenia fornicis. The external sulcus is very shallow and broad.

The habenular ridge is prominent, extending on the ventricular surface of the diencephalon just below the tela chorioidea diencephali from the region of the velum transversum to the epiphysis (fig. 22, *n.h.*). The ridge extending from the posterior end of the habenula to the tegmentum is also prominent (fig. 22, *f.r.*).

The most striking feature, probably, is the great thickening of the posterior extremity of the thalamus. The anterior extremity of the thalamus extends well into the foramen interventriculare, some distance anterior to the velum transversum.

The sulcus limitans is indicated on figure 22 by a dotted line. It is deepest below the posterior pole of the thalamus. Behind the lamina terminalis it is relatively shallow.

The hypothalamic region is marked mainly by its great length. The mammillary recess is indicated (fig. 22, *r.m.*); the floor plate is wide and thin. The infundibular recess (fig. 22, *inf.*) is near the optic chiasm but is separated from it by an unmistakable postoptic recess (fig. 22, *r.post.*). There is no clear external division between hypothalamus and subthalamus. ♦

DISCUSSION

1. *Telencephalon*

a. Recessus preopticus. In all three embryos there is no doubt about the identity of this recess.

b. Velum transversum. Just as obvious is the location of the velum transversum. It is marked by the groove running across the roof plate joining the anterior ends of attachment of the tela chorioidea diencephali to the thalamus. At the lateral end of the velum transversum the taenia thalami meets the taenia chorioidea (fig. 26) and at this point the velum transversum becomes continuous with the angle between the taenia chorioidea and the lateral thalamic wall. In figure 26 an arrow lies in the angle between the taenia chorioidea and the lateral thalamic wall and continues in the velum transversum. The position of the head of the arrow in the mid-line is shown in figure 25. It was noted in the account of the 19 mm. embryo and appears more clearly in the 32 mm. embryo, that if this angle be followed backward it passes behind the attachment of the hemisphere to the thalamic wall, and then as a diagonal groove downward and forward across the lateral wall of the hypothalamus and ends at the optic chiasm. This is the di-telencephalic groove (figs. 14 and 19, *d-t.gr.*) of Johnston.

With the preoptic recess and the velum transversum fixed, the extent of the telencephalic roof plate is determined. Between the preoptic recess and the velum transversum should appear the lamina terminalis, recessus neuroporicus, lamina supraneuroporica, recessus superior, tela chorioidea telencephali medii, and paraphysis.

c. Paraphysis. Just in front of the velum in each embryo is a small arch (figs. 18, 20 and 25, *p.a.*) relatively largest in the 19 mm. embryo, and smallest in the 32 mm. embryo. In each case it lies immediately in front of the velum transversum. In each case also the lateral choroid plexuses arise from its sides. There can be no doubt that this is the paraphysal arch. No indication of the development of a glandular structure could be found. The resemblance of the paraphysal arch in

the 19 mm. embryo to those of the 10 mm. cat and 20 hr. chick figured by Tilney ('15) and of a 4 mm. embryo of *Platydictylus mauritanicus*, figured by Tandler and Kantor ('07), is rather striking.

d. Recessus neuroporicus. An identification of this point is absolutely essential to a final definition of the boundary between lamina terminalis and lamina supraneuroporica. By recessus neuroporicus is meant the most caudal point, i.e., last point, of closure of the neuropore. In many vertebrates at this point a recess appears on the ventricular surface of the roof plate (Johnston, '09).

It was found impossible to identify with certainty this point in the embryos examined. In the 19 mm. embryo, and the 28 mm. embryo, no such recess is apparent. In the 32 mm. embryo, there has been noted just in front of the pit in the roof plate, a shallow notch on the ventricular surface (fig. 25, *r.n.?*). But there is no evidence that this is the recessus neuroporicus, since no such notch appears in either younger embryo.

e. Lamina terminalis. The upper end of the lamina terminalis, as defined by Johnston, has not been determined because the recessus neuroporicus is not apparent. Concerning the major portion of the lamina, however, there can be no doubt. The thick lamina above the recessus preopticus is unmistakable (figs. 18, 20 and 22, *l.t.*).

f. Tela chorioidea telencephali medii. Just in front of the paraphysal arch in the 28 mm. embryo, and the 32 mm. embryo, the roof becomes a single layer of flattened cells (fig. 5, *t.c.t.m.*). This is certainly tela chorioidea telencephali medii. The identification of this tela in the 19 mm. embryo is not so easy. However, I am inclined to identify the summit of the greater arch of the roof plate (fig. 18, *r.s.*) as the recessus superior. This would make the roof between this point (fig. 18, *r.s.*) and the paraphysal arch, tela chorioidea telencephali medii (fig. 18, *t.c.t.m.*). The roof plate here is somewhat thinner than the lower limb of the greater arch (fig. 18, *l.s.?*) and the adjacent hemisphere wall is considerably thinner. The angulus terminalis

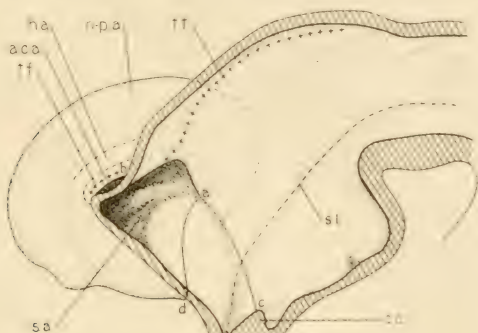
of His probably represents all the membranous parts anterior to the velum transversum.

g. Lamina supraneuroporica. Between what is obviously lamina terminalis and what is just as obviously tela chorioidea telencephali medii in the 28 mm. embryo and the 32 mm. embryo, lies the peculiar thickening of the roof plate before noticed (fig. 6, *l.r.-p.*) which seems by a process of elimination to be lamina supraneuroporica. Whether this interpretation is correct, I cannot say, because I have not had material with which to follow this region in its later development. There is in the 32 mm. embryo, a short portion of the roof plate between the deep pit (fig. 25, *d.p.*) and the shallow recess on the ventricular surface (fig. 25, *r.n.?*) which may be lamina supraneuroporica. In the 19 mm. embryo, the lower limb of the greater arch (fig. 18, *l.s.?*) seems its most likely location.

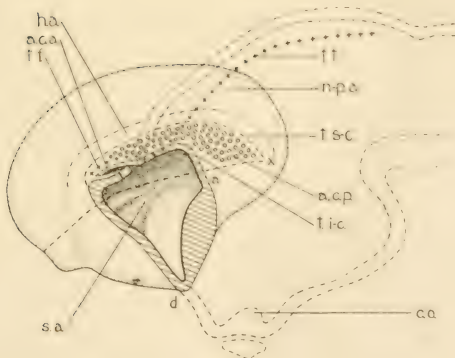
h. Plexus chorioideus ventriculi lateralis. The problem of the formation of the lateral choroid plexuses is one of considerable difficulty, because of the complex morphological relations involved, but if a few facts of development be remembered, the problem becomes relatively simple.

If the brain of a half-grown frog tadpole be examined, it will be found that "the membranous roof of the forebrain ventricle is attached to the massive wall of the hemisphere by the taenia fornicis which is directly continuous caudad with the taenia thalami" (Herrick, '10). The taenia thalami is the attachment of the roof plate posterior to the velum transversum to the lateral wall of the thalamus. It might be added also that the taenia fornicis becomes continuous with the taenia thalami at the lateral end of the velum transversum. In the middle of the membranous roof in front of the velum transversum arises the paraphysis. There is no plexus lateralis in the larval or adult frog, but in urodele Amphibia it is between the paraphysis and the taenia fornicis that the lateral choroid plexus makes its appearance, pushing into the ventricle. Warren ('05) in describing the development of the lateral plexuses of *Necturus maculatus* says, "The telencephalic plexus develops from the

the taenia fornicis from the area chorioidea posterior, that is, between the taenia fornicis and the mid-dorsal line, is placed another area of circles labeled area chorioidea anterior. It is



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17

Fig. 16 Median view of a model of the forebrain in His' 13.6 mm. embryo, CR.

Fig. 17 Hypothetical view of the median wall of the cerebral hemisphere from His' 13.6 mm. embryo, CR. The hemisphere has been excised along the line *b-a-d* in figure 16.

in this latter region that the lateral choroid plexus makes its appearance in urodele Amphibia as we have just remarked, and here also it makes its first appearance in the human embryo. The hippocampal area is not apparent in this embryo but later developments show that its anlage must lie in some such position as indicated in figure 15. The part of the telencephalon which is evaginated to form the cerebral hemisphere is the part anterior to the line *d-a-b*. The division between telencephalon and diencephalon is represented by the line *b-a-c*.

Keeping these relations in mind, we may easily understand His' embryo CR, 13.6 mm. in length. Figure 16 shows a medial view of the forebrain. An invagination of the telencephalic roof has taken place between the taenia fornicis and the mid-line, forming the fissura chorioidea in the region which was marked area chorioidea anterior in figure 15. The hemisphere vesicle has enlarged mainly by enlargement and evagination of the neo-pallial area (fig. 15, *n-p.a.*), so that the hippocampal area which in figure 15 lay below the roof plate and in front of the area chorioidea posterior, now lies above the roof plate and behind the area chorioidea posterior, and its ependymal face has now come to look lateralward. So also with the area chorioidea posterior, which lies now in the evaginated cerebral hemisphere behind the di-telencephalic groove, and its ependymal face is now its lateral face. When the hemisphere evaginated, the wall bent for the most part along the line of the taenia fornicis in figure 15, the di-telencephalic groove as far as the point *a*, and then along the line *a-d*.

If now the hemisphere in embryo CR be excised along a line homologous with the line *b-a-d* in figure 15, the relations of the area chorioidea posterior and area chorioidea anterior are more clearly seen. The medial surface of such a hypothetical hemisphere is represented in figure 17. The area chorioidea anterior has invaginated into the lateral ventricle. The resulting plexus has been cut off in figure 17, leaving the fissura chorioidea. Since the invagination takes place between the taenia fornicis and the mid-line of the telencephalic roof, the taenia fornicis lies along the lateral and upper margin of the fissura

chorioidea, or in other words, along the line of attachment of the plexus to the medial hemisphere wall. If, therefore, we wish to follow the taenia fornicis et thalami in the embryo CR, we must follow the line marked with crosses in figures 16 and 17 along the wall of the diencephalon (figs. 16 and 17, *tt.*), across the velum transversum, and then between the area chorioidea posterior and area chorioidea anterior above and lateral to the fissura chorioidea (figs. 16 and 17, *tf.*).

It will be seen (fig. 17) that the area chorioidea anterior lies entirely anterior to the velum transversum. The lateral choroid plexus never approaches the mid-line except along the sides of the paraphysal arch, anterior to the velum transversum. The area chorioidea posterior has relations entirely analogous with its relations in figure 15, except for its change of face. It lies between the hippocampus and the di-telencephalic groove, and adjoins the posterior end of the taenia fornicis on the upper side, which in figure 15, was the lower side of the taenia.

This stage in the process bears a close resemblance to the condition in the brain of *Platydactylus mauritanicus*, as described by Tandler and Kantor ('07). Concerning the development of the structures in the region of the foramen of Monro, they write (*italics mine*):

Die zwischen den beiden Foramina Monroi gelegene Decke des Ventriculus impar [telencephalon medium] ist rein epithelialer Natur und *grenzt sich lateralwärts durch je eine deutliche Furche Sulcus tegmenti ab.*

The lateral edge of this sulcus tegmenti is the taenia fornicis.

Die Fissura chorioidea, welche von hinten her den Raum des Foramen Monroi einengt, *entwickelt sich, wie die Durchsicht der Serie lehrt, derart, dass sie im vorderen Abschnitt aus dem Sulcus tegmenti selbst, hinten aber oberhalb dieser Furche entsteht, und hier die mediale Hemisphären wand einschneidet.*

Der Plexus chorioideus des Ventriculus lateralis stülpt nämlich *nur ein ganz kurzes Stück der Hirnwand ein. Die Einstülpungsstelle selbst, liegt wie man um Stadium V zeigen kann, gerade dort, wo das hintere Ende des Sulcus tegmenti die Decke des Telencephalon impar von der medialen Hemisphärenwand absetzt.*

Here again, as in the brain of *Necturus maculatus*, the half grown frog tadpole, and His' embryo CR, the taenia fornicis can be followed along the lateral margin of the sulcus tegmenti past the velum transversum to the taenia thalami. But when the sulcus tegmenti invaginates to form the fissura chorioidea, the posterior end of the taenia fornicis and a small part of the medial hemisphere wall are drawn in also. This latter process is carried much farther in the human embryo.

In the evagination of the hemisphere in the human embryo, the point marked *x* in figure 15, at the junction of the hippocampal area, striatal area, and area chorioidea posterior, remains always at the posterior pole of the hemisphere. The result on the corpus striatum, as the hemisphere extends backward, is to draw out the tail of the caudate nucleus. The result upon the area chorioidea posterior is to draw it out in a thin lamina marked in figure 17 by small circles.

The area chorioidea posterior now buckles into the lateral ventricle, and we have the condition found in the 19 mm. embryo, H 173. The area chorioidea posterior is clearly shown by figure 19, being all of the plexus back of the point marked *z*, and has buckled inward only slightly, leaving a very wide fissura chorioidea. The point *z* lies opposite the velum transversum.

It thus appears that the lateral choroid plexus is composed of two parts, a pars anterior plexus chorioidei ventriculi lateralis which is formed by the invagination of the area chorioidea anterior, between the paraphysal arch and the taenia fornicis, and a pars posterior plexus chorioidei ventriculi lateralis formed by the infolding of the area chorioidea posterior in the medial wall of the hemisphere.

If the taenia fornicis be now followed in the 19 mm. embryo, it will not be found to become continuous with the taenia thalami because the area chorioidea posterior, to which it was attached toward its posterior end, has now buckled into the ventricle. If, therefore, we follow the attachment of the lateral choroid plexus to the medial hemisphere wall, we follow the taenia forni-

cis as far as the area chorioidea posterior, then along the upper margin of the area chorioidea posterior (anterior margin in fig. 15) called also taenia fornicis in adult anatomy, around its posterior extremity (lower extremity in fig. 15), and back along its lower margin (posterior margin in fig. 15) called also taenia chorioidea, to the lateral end of the velum transversum where we finally reach the taenia thalami.

It thus appears that the portion of the taenia fornicis of human anatomy which lies adjacent to the pars posterior plexus chorioidei ventriculi lateralis is not homologous to the taenia fornicis in Anura and would better be called taenia suprachorioidea, and the taenia chorioidea correspondingly termed taenia infrachorioidea. The illustrations have been labeled in accordance with established usage. To place them in accord with the foregoing conclusions, in figures 1, 11, 12, 18, 24 and 26 taenia fornicis should be changed to taenia suprachorioidea, and taenia chorioidea to taenia infrachorioidea.

In later stages the taeniae infra- et suprachorioidea become approximated closely as is found in the 28 mm. embryo and in the 32 mm. embryo, and the fissura chorioidea is reduced to a narrow slit, its axis in the plexus of the 19 mm. embryo, lying probably along the dotted line in figure 19.

In the 28 mm. embryo and the 32 mm. embryo, it is impossible to distinguish the dividing line between the pars anterior and the pars posterior of the lateral choroid plexus, and the taenia fornicis in its restricted sense is relatively of very short length.

i. Sulcus limitans. The sulcus limitans is lost in a deep recess between the corpus striatum and hypothalamus. This recess has disappeared by fusion of its walls in the 32 mm. embryo. If the sections of the 19 mm. embryo be followed, the beginning of this process can be readily seen (fig. 2).

j. Corpus striatum. There is nothing extraordinary about the corpus striatum in either embryo. The approximation of the thalamus and corpus striatum in the foramen interventriculare (fig. 22) is of interest when one remembers Goldstein's work. Of course, the entire connection between the thalamus and

corpus striatum is not formed by fusion, and there is as yet no fusion here. The intermediate root of the corpus striatum probably extends into the medial hemisphere wall, but the external morphology does not suggest it.

2. *Diencephalon*

a. *Tela chorioidea diencephali*. It is to be noted that the tela chorioidea diencephali shows no indications of folding except at its anterior extremity. In the 32 mm. embryo, a pouch arises at the anterior end and extends forward over the velum transversum and the paraphysal arch (fig. 25, *a.p.*). Streeter, in Keibel and Mall's textbook, as was mentioned in the history, makes the statement that "Orally this choroid roof [of the third ventricle] is continued into the telencephalon where it forms a pointed pouch overlapping the lamina terminalis and the contained commissures. . . . The anterior choroidal pouch has been homologized with the paraphysis of the lower vertebrates." There is not up to this stage any pointed pouch in the telencephalic roof. The pouch noted above lies in the roof of the diencephalon, just back of the velum transversum and hence cannot be the paraphysis. The true paraphysal arch has been pointed out above. In comparing Francotte's figures with this region in embryos of approximately the same age in the Chicago collection, I feel sure that it was this anterior pouch of the tela chorioidea diencephali which he described as the paraphysis.

I have not followed the thalamic lip fully in later stages, but in an embryo of 60.4 mm. greatest length, H 44 of the Chicago collection, the thalamic lip is fused with the lateral thalamic wall toward the anterior end.

b. *Epiphysis*. The epiphysis, in the 19 mm. embryo especially, shows marked indications of a differentiation into epiphyseal stalk and epiphyseal vesicle. Such a condition is very characteristic of lower vertebrates, especially reptiles, but has never before been noticed in the human embryo. The epiphyses of the 28 mm. and 32 mm. embryos show similar indications.

In the 32 mm. embryo and in the 28 mm. embryo, the tela chorioidea continues on to the epiphyseal evagination. A similar condition has been noted in the cat by Thompson ('09).

c. Habenula. The character of the ridge running from the anterior end of the habenula to the subthalamus in the 19 mm. embryo (fig. 18, *h.s-l.r.*) is not apparent. It is probably only a temporary fold mechanically produced by inequalities in development of the thalamic wall. It has entirely disappeared in both older embryos.

The ridge extending from the posterior end of the habenular prominence in the 28 mm. embryo and the 32 mm. embryo (figs. 20 and 22, *f.r.*) indicates the position of the fasciculus retroflexus (Meynerti).

d. Thalamus. In the 19 mm. embryo, only the anterior, inferior part of the thalamic wall (fig. 18, *th.f.*) lying between the sulcus limitans and the habenulo-subthalamic ridge is thickened. In the 28 mm. embryo, the ridge has disappeared and the anterior portion of the thalamic wall which lay above the ridge is thickened (figs. 18 and 20, *th. 2*). This region probably contains the principal nuclei of the thalamus. The posterior extremity of the thalamus (figs. 18 and 20,) is still somewhat flattened and thin in the 28 mm. embryo, but becomes very thick in the 32 mm. embryo (fig. 22).

e. Sulcus limitans. There is no difficulty in following the sulcus limitans. In the 32 mm. embryo under the posterior pole of the thalamus, it is very deep.

f. Hypothalamus. The hypothalamus in all these embryos is of great antero-posterior extent. It will be noticed also that the infundibulum, especially in the 19 mm. embryo, lies a considerable distance back of the optic chiasm. It seems quite probable that, as Johnston ('09) has remarked, in His' model of the embryo CR of 13.6 mm., the recess marked infundibular is really postoptic, and the real infundibular recess lies back of it and is labelled tuber cinereum. Only in the 19 mm. embryo is the subthalamus clearly separated externally from the hypothalamus.

SUMMARY

1. The plexus chorioideus ventriculi lateralis is composed of two distinct portions, of which the anterior is developed from the roof plate in the angle between the paraphysal arch and the medial wall of the hemisphere, and the posterior from that part of the medial wall of the hemisphere just anterior to the di-telencephalic groove and homologous to the anterior limb of the velum transversum.

2. The taenia fornicis of adult human anatomy, except for its extreme anterior end, is not homologous with the taenia fornicis of Anura.

3. The position of the recessus neuroporicus could not with certainty be ascertained, and identification of the lamina supra-neuroporica was therefore uncertain.

4. The tela chorioidea telencephali medii is present in the embryos of 28 and 32 mm.

5. The paraphysal arch can be followed to the embryo of 32 mm., as an arch of the roof plate of the telencephalon. It lies just anterior to the velum transversum and from its sides arise the lateral choroid plexuses. The anterior pouch of the choroid plexus of the third ventricle lies in the diencephalon and is not, therefore, homologous to the paraphysis of the lower vertebrates. No indication of the development of a glandular structure was found.

6. The velum transversum can be traced up to the 32 mm. embryo, joining the anterior extremities of attachment of the tela chorioidea diencephali to the lateral thalamic wall, and its groove is continuous laterally with the angle between the taenia chorioidea and the lateral thalamic wall, the di-telencephalic groove.

7. The tela chorioidea diencephali is not folded nor vascularized except for its extreme anterior end in the embryo of 32 mm. It is broadened by the formation of a thalamic lip, very much resembling the rhomboidal lip of the rhombencephalon.

8. The epiphyses of all three embryos show indications of the presence of the homologue of the pineal vesicle of the lower

vertebrates. In the embryos of 28 and 32 mm., the tela chorioidea diencephali is continued on to the epiphyseal outgrowth.

9. The position of the fasciculus retroflexus (Meynerti) is indicated in the embryos of 28 and 32 mm. by a pronounced ridge.

10. There is evidence that the connection of the corpus striatum and thalamus is thickened by fusion of the medial root of the corpus striatum with the anterior extremity of the thalamus in the foramen interventriculare.

11. The sulcus limitans is lost in a very deep recess in the embryo of 19 mm. between the corpus striatum and hypothalamus, and this recess disappears in later stages by fusion of its walls.

12. The length of the hypothalamus in embryos from 19 to 32 mm. is relatively very great. In the embryo of 19 mm. the subthalamus is separated externally from the hypothalamus.

13. The infundibular outgrowth is still some distance back of the optic chiasm at 19 mm. and becomes shifted nearer only in much later stages.

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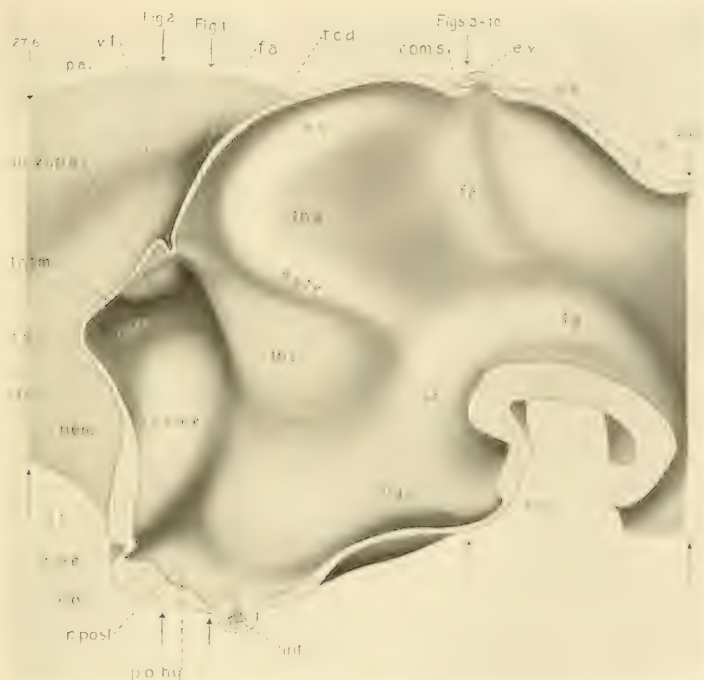
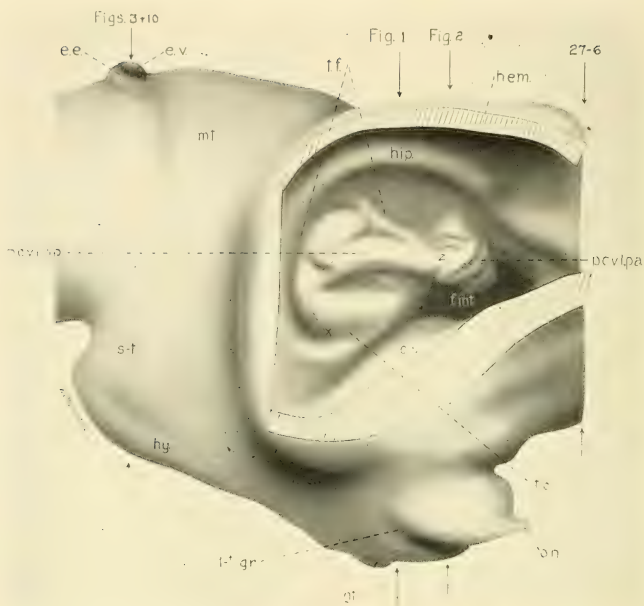


Fig. 18. Median view of a model of the forebrain from the 19 mm. embryo, H 173. $\times 22\frac{1}{2}$. The dotted line follows the sulcus limitans. The limiting membrane bounding the epiphyseal vesicle, *e.v.*, ventrally in this figure should be represented as incomplete; cf. figure 10.

In this and some of the following figures the planes of the cross sections in the text-figures are indicated. The arrows at the right and left ends of the models are marked with the slide and section numbers of the first and last sections represented in the models. The reference letters for this and the following figures are found on page 88.



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Fig. 19 Lateral view of a model of the forebrain from the 19 mm. embryo, H 173. $\times 22\frac{1}{2}$. The lateral wall of hemisphere has been cut away, exposing the lateral choroid plexus. Median surface of this model is shown in figure 18.

Fig. 20. Median view of a model of a portion of the forebrain from the 28 mm. embryo, H 91. $\times 18$.

Fig. 21 Lateral view of a model of a portion of the forebrain from the 28 mm. embryo, H 91. $\times 18$. The lateral hemisphere wall and corpus striatum have been cut away, exposing the lateral choroid plexus. Median surface shown in figure 20.

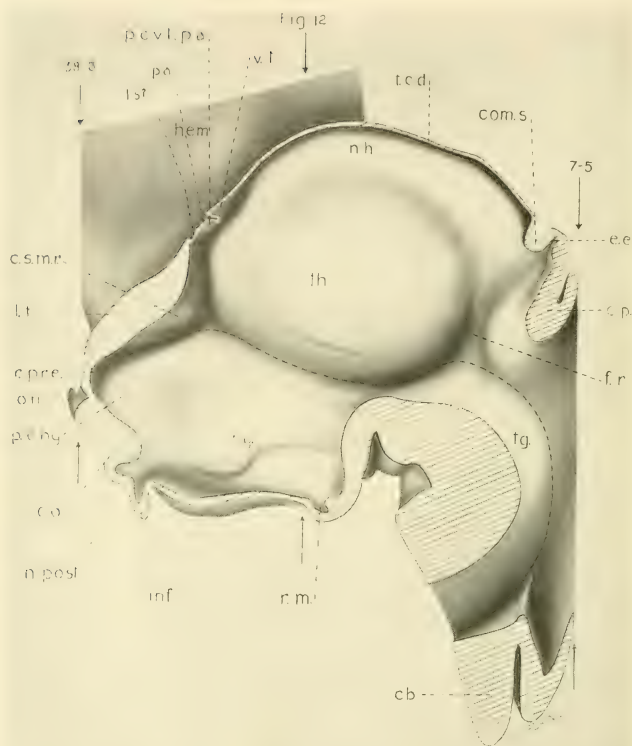


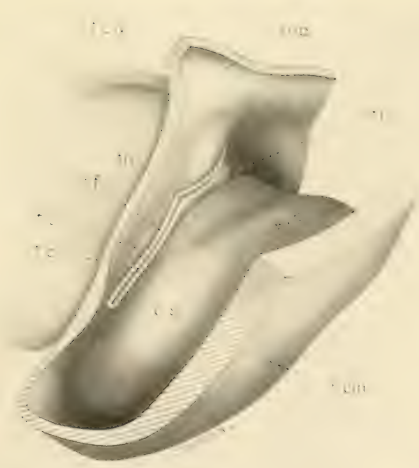
Fig. 22 Median view of a model of the forebrain from the 32 mm. embryo, H 41. $\times 12\frac{1}{2}$. The dotted line follows the sulcus limitans.

Fig. 23 View of the anterior end of the model of the forebrain from the 19 mm. embryo, H 173, shown in figure 18. $\times 22\frac{1}{2}$. View taken slightly from the medial side and above. A portion of the median hemisphere wall and lamina terminalis has been removed exposing the corpus striatum.

Fig. 24 View of the posterior extremity of the corpus striatum, in the model of the forebrain of the 32 mm. embryo, H 41, shown in figure 22. $\times 12\frac{1}{2}$. View taken from above, behind and lateralward.



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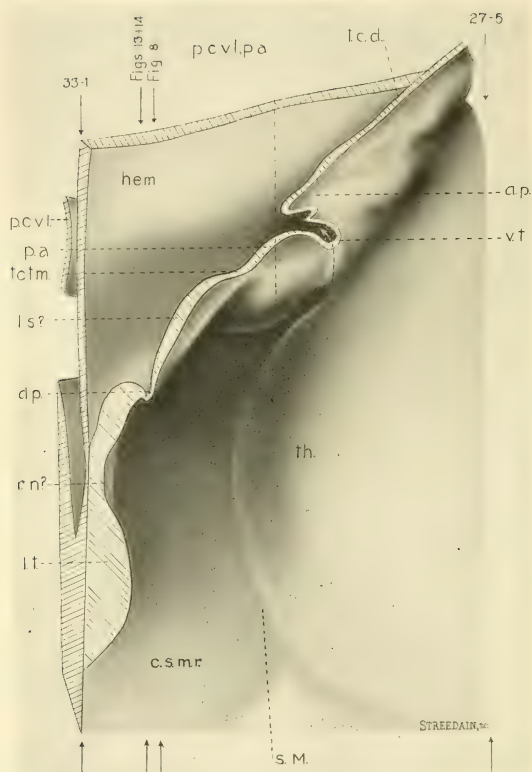


Fig. 25 Median view of a model of the region around the foramen intertricular from the 32 mm. embryo, H 41. $\times 50$. (The entire forebrain of this embryo is shown in figure 22.) The model was made at a magnification of 100 diameters. Anterior end to the left.

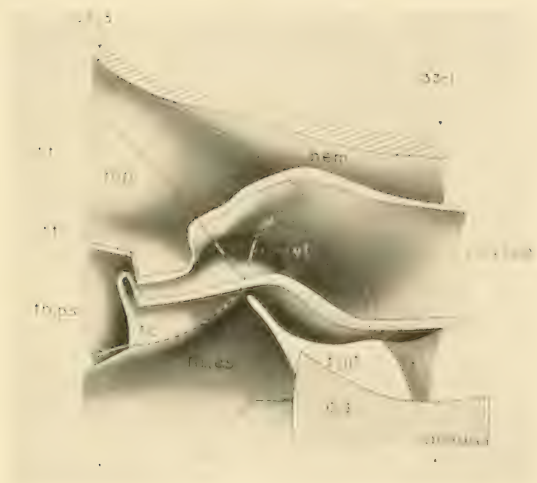


Fig. 26 Lateral view of a model of the region around the foramen inter-ventriculare from the 32 mm. embryo, H 41. $\times 50$. Median view of the same model is shown in figure 25. For help in orientation, the approximate position of the roof plate, which lies on the opposite side of the hemisphere wall, has been projected through as a dotted line. The location of the taenia thalami is indicated by a row of dots and dashes. An arrow lies in the di-telencephalic groove and continues across in the groove of the velum transversum. The position of its head may be seen by reference to figure 25. Anterior end of model to the right.

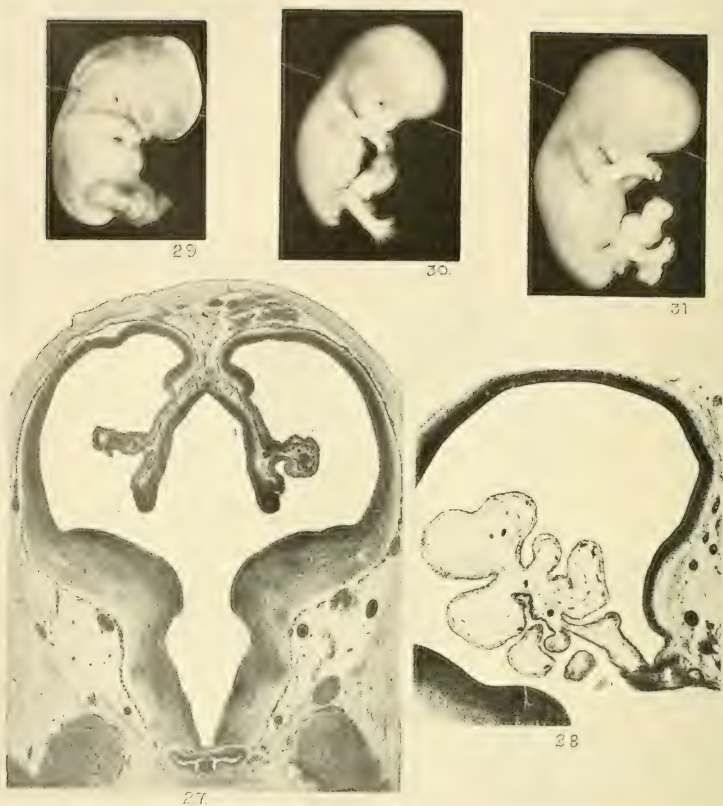


Fig. 27 Photograph of a section through the diencephalon of the 19 mm. embryo, H 173. $\times 16\frac{3}{4}$. Slide 21, Sect. 11. The break in the hemisphere wall to the left is an injury to this individual section. Compare figure 1, drawn from the same section.

Fig. 28 Photograph of a section through the lateral choroid plexus of the 28 mm. embryo, H 91. $\times 20$. Sect. 355. Section taken midway between figures 7 and 11, as shown in figure 20.

Fig. 29 Photograph of the 19 mm. embryo, H 173. $\times 2$. Plane of section indicated.

Fig. 30 Photograph of the 28 mm. embryo, H 91. $\times 1\frac{1}{4}$. Plane of section indicated.

Fig. 31 Photograph of the 32 mm. embryo, H 41. $\times 1\frac{1}{4}$. Plane of section indicated.

THE MOVEMENTS IN THE VISUAL CELLS AND RETINAL PIGMENT OF THE LOWER VERTEBRATES

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ONE TEXT FIGURE AND FIVE PLATES

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1. INTRODUCTION AND HISTORICAL REVIEW

The positional changes that occur in the vertebrate retina chiefly through the action of light have attracted the attention of many workers in continental Europe although, strangely enough, neither English nor American investigators have hitherto concerned themselves with this particular field of endeavor. The results acquired from these researches have proved of such interest and significance as to equal, and perhaps even to sur-

pass, those obtained from the two other branches of retinal physiology, namely, the study of the visual purple and of the action currents in the optic nerve.

In order that the reader may understand the present status of this subject, it is desirable to summarize briefly the general conclusions that have been established relative to the efficiency of light in producing movements in the retinal elements. For a more extensive review of this literature reference may be made to a separate paper by the writer (Arej, '15) or to the excellent compilation of the German author, Garten ('07).

Although a variability in the position of the retinal pigment had been noted by early workers (H. Müller, '56; Morano, '72) the cause remained unsuspected until Boll ('78) and Kühne ('77) independently discovered that in the light the retinal pigment of the frog extends nearly to the external limiting membrane whereas in darkness it retreats, thereby forming a compact layer next to the choroid¹ (figs. 1 and 4). Later observations have corroborated Kühne's original view that pigment migration is not due to the extension and retraction of cell processes but to the movement of pigment granules in the protoplasm of relatively fixed cells.

The most extensive pigment migration occurs in fishes (Stort '86) and in anuran amphibians (Boll, '78, and Kühne '77, on the frog; Arcoleo, '90, on the toad), whereas the positional changes in the pigment of urodeles are relatively limited (Angelucci, '78). Well defined movements of the retinal pigment are also found among birds, including not only those that are diurnal, but also some that are nocturnal in habit. Among reptiles (Angelucci, '90) and mammals (Angelucci, '78) limited pigment changes have probably been detected, notwithstanding the contradictory evidence presented by various workers.

¹ Czerny ('67) found that when sunlight was concentrated by a lens on the retinas of various animals, the pigment became more highly expanded in the affected regions than in other portions which had been exposed to light but not so treated. In his experiments no comparisons were made with dark-adapted retinas. These somewhat pathological tests were not well substantiated by the later work of Deutschmann ('82).

Movements of the inner member of the vertebrate cone cell were first observed by Stort in 1884, although the earliest announcement of this discovery was published by Englemann ('85) in whose laboratory Stort worked. To the contractile portion of the cone's inner member Englemann applied the significant term 'myoid' (figs. 30, 31; *my. con.*). The contractility of the myoid is extraordinary, since in some fishes light produces a shortening of this part to 10 per cent of the length which it assumes in darkness (figs. 25, 27). If effective at all, light always causes a shortening and darkness an elongation of the cone cell.

Stort ('87) extended his first discovery on the frog by experimentation upon representatives of the various other vertebrate classes, thereby showing that in fishes and birds extensive movements of the cones likewise occur. In the salamander, as a type of urodele amphibian, responses of the cones to light have both been asserted (Angelucci, '90), and denied (Garten, '07). Among a few reptiles (Englemann, '85) and mammals (Garten, '07) slight changes have apparently been detected.

The visible response of the rod's inner member presumably is not identical throughout all the vertebrate classes. Angelucci ('84) was the first to observe a shortening of the frog's rod after exposure to light and later, in 1890, he applied the term 'myoid' to the contractile portion of the rod's inner member (figs. 30, 31; *my. bac.*) in a sense similar to that in which Englemann has previously used it for the corresponding portion of the cone; Areoleo ('90) likewise reported that the rod myoid of the toad shortens in the light. Recently, however, Lederer ('08) has asserted that the photomechanical change in the rod myoid of the frog is not a shortening but an elongation. In all fishes that possess cones, the rod myoid lengthens in the light and shortens in darkness, as Stort ('87) first believed. The response in the rods of day-birds (Stort, '86) is similar to that in fishes, although in night-birds (Garten, '07) movements of these cells may be entirely absent. No experimentation has been performed upon the rod cells of mammals or of reptiles; in the latter group, however, the retina usually lacks these elements.

2. MATERIAL AND TECHNIQUE

The work of Chiarini ('04a, '06), in which he systematically investigated the effect of light on the retinas of representative vertebrates, led him to the conclusion that the maximal changes in the position of the pigment and of the cone cells occur in the lower vertebrates and particularly in the fishes, whereas in the highest vertebrates these changes are extremely limited or may even be imperceptible. For experimentation involving variable temperature poikilothermous animals are necessary, and no vertebrates are more easily available for this purpose than are fishes and amphibians. When to availability is added the advantages which they offer by the possession of exceptionally well developed visual and pigment cells, which are capable of undergoing the greatest dimensional changes found in any of the vertebrates, it will be seen that these animals are particularly favorable for such physiological studies as were conducted in the present investigation.

The following fishes were used: the common horned pout (*Ameiurus nebulosus* Lesueur); the common killifish (*Fundulus heteroclitus* Linn.); the shiner (*Abramis crysoleucas* Mitchill); and the goldfish (*Carassius auratus* Linn.). Of these, all but *Fundulus* are fresh water forms, while *Fundulus* is found in brackish water, and especially at the mouths of fresh water streams. *Abramis* and *Carassius* belong to the same family, the others to different families. Goldfish were obtained for the most part from dealers although a number of feral animals were used. Many of the streams and ponds about Cambridge are stocked with feral goldfish, liberated by accident or design. The majority of such animals have lost their gold coloration and have returned almost completely to the original olivaceous type.

The amphibians used were as follows: the leopard frog (*Rana pipiens* Schreber); the green frog (*Rana clamitans* Latreille); the bull frog (*Rana catesbiana* Shaw); and the mud puppy (*Necturus maculatus* Raf.). Of the three species of frogs, the leopard frog was used almost exclusively for experimentation on adult animals; the larvae only of the bull frog were employed.

The technique of preparing retinas for microscopical examination was very simple. Eyes were removed from their orbits in two ways. In *Ameiurus*, where the eyes are prominent and the skin is soft, excision was performed directly. The eyes of amphibians and of fishes other than *Ameiurus*, particularly in experiments conducted in the dark where rapidity of operation was desirable, were not excised directly but according to the following procedure. With heavy scissors the cranium was bisected in a median sagittal plane; following this, a transverse cut just posterior to the orbit freed the two halves of the cranium, with the contained eyes, from the rest of the body. In either case the operation was performed in a few seconds and the eye, without being handled, was allowed to drop into the fixing fluid.²

Both Perenyi's and Kleinenberg's fluids gave good fixation (Howard, '08; Palmer, '12), but of the two, Perenyi was preferred. The toughness of the sclera and the consequent slowness in the penetration of fluids demands generous allowances of time during the various steps preparatory to imbedding in paraffine. Dehydration and clearing in xylol should, however, progress as rapidly as possible since otherwise the sclera becomes extremely hard.

Two methods were used in removing the lens, one of which, although longer, gave much more satisfactory results. The first somewhat tedious procedure consisted in paring away the front face of the eyeball with a razor after the eye had been previously imbedded in paraffine. After removing the face of the eyeball slightly beyond the ora serrata, the lens was pried from its paraffine matrix with a dissecting needle; following such manipulation reimbedding was of course necessary. The second and simpler method was to remove the face of the eyeball with fine curved scissors after the eye had been hardened in absolute alcohol; if, however, the eye was not sufficiently hardened or the

² A method of simple decapitation used by Pergens has been criticized by various workers who contend that slight changes occur in the position of the retinal elements after the head is immersed in the killing fluid. In a series of controlled experiments, however, I could detect no post-mortem disturbances when the head was both bisected and cut from the trunk.

greatest care was not exercised, the retina proper easily separated from the pigmented epithelium. On the whole, the first method was preferred to the second because of the wrinkling of the retina that usually accompanied the use of the latter.

Sections were cut $7\ \mu$ to $10\ \mu$ thick, and except in a few special cases only those passing through the region of the optic nerve were retained for examination. Preparations were stained with Ehrlich-Biondi's triple stain or were double stained in Heidenhain's iron hematoxylin and a plasma counterstain. Ehrlich-Biondi in some instances gave excellent differentiation of all elements, while at other times it would show the capriciousness in producing satisfactory results for which it is notorious; iron hematoxylin gave uniformly good preparations.

It often became necessary to bleach the pigment in order to study the visual cells, which would otherwise be masked by the partially or completely extended processes. The method employed was essentially that of Mayer ('81), in which nascent oxygen³ is the effective agent.

The aim of the present investigation has been to determine the influence of light, temperature, anaesthetics and oxygen on the movements of the rods, cones, and retinal pigment in the normal and excised eyes of fishes and of amphibians.

To Prof. G. H. Parker, under whose direction this research has been conducted, I wish to acknowledge my indebtedness for much inspiration and valuable suggestion. Advantage is taken of this opportunity to express appreciation for the facilities of the Zoölogical Laboratory placed at my disposal by the Director, Prof. E. L. Mark, and for many courtesies extended by him during my residence at Cambridge.

3. EXPERIMENTAL PART

A. DETERMINATION OF ADAPTION TIMES

Before extensive experimentation can be undertaken on the retinal elements, it is necessary to determine the various lengths

³ When potassium chlorate and hydrochloric acid interact, it is commonly said that nascent chlorine is the agent causing bleaching. As a matter of fact the reaction liberates free oxygen.

of the time which they require in assuming the positions characteristic of light and of darkness. In most cases it is not easy to state definitely when adaption is completed, for the response becomes less vigorous as it nears the end and consequently the factor of personal equation is unavoidable.

Light intensity and temperature (Dittler, '07) represent variables which undoubtedly play a part in the determination of adaption time. No attempt was made to discover the exact rôle of either of these factors, although the general experimental conditions were kept approximately uniform during successive trials.

The effect upon adaption time of a long or short preliminary subjection to light or darkness, has never been taken into account, although such influences were asserted by Gaglio ('94).

a. Retinal pigment

Pergens ('96) found that after 2 minutes' illumination the retinal pigment of *Leuciscus* began to expand. After 1 minute of darkness a noticeable contraction occurred, which was completed in 20 minutes. Chiarini ('04b), working on the same fish, came to somewhat different conclusions. He observed a sensible pigment expansion after direct exposure to sunlight for 1 minute, although complete light adaption necessitated a period of 1 hour. The reverse process of dark adaption was not initiated until the animal had been subjected to darkness for from 4 to 5 minutes, and a minimum of 1 hour was required to complete the contraction.

When the retinal pigment of fishes has undergone a maximum expansion, it accumulates distally⁴ near the external limiting membrane, whereas the proximal portions of the cells are to a greater or less extent devoid of pigment (figs. 9, 10). Such a

⁴ The term 'proximal' as used in this paper will refer to movements toward the nuclei of a given cell, either pigment or visual. In like manner 'distal' will have reference to movements away from the nucleus. Since the distal movement in the pigment cells is the reverse of that in the rods and cones, an arbitrary nomenclature with reference to the eye-ball becomes confusing, while the terminology here suggested lends itself readily to descriptions of the moving parts.

condition of distal accumulation requires additional time after the 'front rank' of pigment granules has arrived at the position of maximal extension. In the determinations made by me, light adaption was not considered to be completed until the pigment was thus maximally expanded.

The results obtained on the retinal pigment of fishes were as follows:

Ameiurus

Diffuse daylight

30 minutes, pigment incompletely extended

40 minutes, pigment fully extended, but homogeneously distributed

1 hour, maximal expansion

Darkness

45 minutes, pigment about half contracted

1 hour, maximal contraction

The movements of *Abramis* were more rapid, notwithstanding an apparently heavier pigmentation of the retina.

Abramis

Diffuse daylight

15 minutes, lightly pigmented processes three quarters extended. Most of the pigment at the bases of the cells

30 minutes, processes fully extended. Distal pigment accumulation begun

45 minutes, maximal expansion

Darkness

20 minutes, pigment about half contracted

30 minutes, maximal pigment contraction

The results of the dark adaption on *Fundulus* were less satisfactory, since the delimitation of the pigment in darkness is poorly defined.

Fundulus

Diffuse daylight

45 minutes, pigment fully extended; but little tendency exhibited towards distal accumulation

1 hour, maximal expansion

Darkness

45 minutes to 1 hour, maximal contraction

As regards the adaption time in the frog, Stort ('87) believed that in 1 hour of bright, diffuse daylight or in 4 hours of dark-

ness, maximal light- and dark-adaption of the pigment respectively was produced. Kühne ('79) stated that complete dark-adaption occurred in 1 to 2 hours, this period corresponding to the time necessary for the regeneration of the visual purple. Chiarini ('04b) maintained that following an exposure of half an hour to direct sunlight one and one-half hours were needed to complete the adaption in darkness. Although no direct experimentation was made on the frog, the general experience gained from working with this animal leads me to suspect that 1 hour for light-adaption and 1 to 2 hours for dark-adaption are approximately the proper amounts.

b. Visual cells

Relative to the adaption times of the cone cells, Pergens ('96) stated that in *Leuciscus* the first visible shortening occurred after an exposure to light of 1 minute. According to his illustrations, the cones were very much shortened after 2 minutes while those that had been subjected to light for 5 minutes were practically in the position characteristic for light. When light-adapted animals were introduced into the dark, a lengthening of the cones was evident after 1 minute and in 5 minutes the elongation was nearly complete, although it did not become maximal for 20 minutes. Pergens' method of allowing decapitated heads to remain in the light during fixation has been criticized by Garten ('07), and the later work of Pergens ('99) has likewise been questioned by Herzog ('05). These workers assert that it is entirely possible that the changes initiated by the action of light continue until the actual penetration of the fluid into the eye fixes the retina. Certain experiments of Weiss, instigated by Garten, indicate that the action of light can influence the position of cones and retinal pigment after decapitated heads have been introduced into the fixative. These results are in opposition to the statement of Chiarini ('04b) that light cannot be effective during fixation.

In an attempt to avoid this source of error, at the completion of an experiment the light-adapted eyes were fixed in exceedingly

dim light. It is probable that this precaution was sufficient, for, as will be shown later, the movements of the cones of *Abramis*, the only fish worked upon, are not rapid and moreover no changes occur even when the excised eyes, immersed in water, are subjected to light or to darkness.

Temperature is an important factor that must be considered in the adaption of cones. In anticipation of certain results that will be found in another part of this paper, it may be said that the cones of fishes are maximally extended at about 25°C. in the dark (fig. 27), and in the case of *Abramis* at least, they are also maximally shortened at 5°C. in the dark (fig. 25). Moreover, as temperature does not affect the length of the cones when they are under the influence of light, the animals may be kept at a temperature of 25°C. during an entire experiment and the resulting movement of the cones will then be solely traceable to conditions of light or darkness.

The results on the cones of *Abramis* may be summarized as follows:

Diffuse daylight

- 15 minutes, cones much shortened—perhaps two thirds
- 23 minutes, approximately the same condition as at 15 minutes
- 30 minutes, shortening not quite complete in most animals
- 45 minutes, maximal light adaption

Darkness

- 13 minutes, cones somewhat extended—one third (?)
- 20 minutes, extension practically complete
- 30 minutes, maximal dark adaption

The adaption times of the cones of *Abramis* are longer than those given by Stort for *Leuciscus*. This, in part, may be due to the wider range between the positions of maximal light- and dark-adaption which was produced by the aid of elevated temperature.

Englemann ('85), working on the frog, was the first to discover that the movements of the cones were not accomplished instantaneously, but required definite periods of time. He also observed that elongation in the dark was a longer process than shortening in the light. My results on *Abramis* do not entirely support his latter view. At first the cones of this animal do

respond more actively when stimulated by light, but a longer time is required to complete the process of light-adaption than the reverse changes in the dark.

No experimentation seems to have been performed upon any animal to determine the adaption time of the rod cells. *Ameiurus* was selected for these tests, for the rods differ greatly from those which are characteristic of fishes in general. Instead of the slender elements $1.5\ \mu$ to $2.0\ \mu$ in diameter, which, for example, are found in *Abramis* (fig. 25), the rods in *Ameiurus* (fig. 31) are robust and resemble more closely those of the frog (fig. 35). The barrel-shaped ellipsoid measures about $4\ \mu$ in either dimension, and the width of the outer membrane is the same. With this can be compared the width of the outer member of the rods in the frog, which in my preparations of *R. pipiens* measured, for the most part, $5\ \mu$, although Howard ('08) states the width as $6\ \mu$, and H. Müller ('56) as from $6\ \mu$ to $7\ \mu$.⁵ The species was not mentioned by either of these writers.

Unlike the cones, the rods of *Ameiurus* in the dark form a more or less even row close to the external limiting membrane (fig. 31), while in the light the myoid elongates carrying the ellipsoid and outer member far up into the pigment layer (fig. 30). The extent of these positional changes may be judged from measurements of rods in darkness and in light which give extreme values of $70\ \mu$ and $7\ \mu$ respectively for the length of the myoid. I know of no fish in which rods of this size have been described, although Gatten ('07) makes particular mention of the pike as possessing 'grosse Stäbchen.' It is evident that the large size and the extensive positional changes which the rods of *Ameiurus* undergo make them especially favorable for physiological experimentation.

The effect of temperature upon the length of the rods is comparatively slight, hence the following determinations on the rods of *Ameiurus* were conducted at room temperature.

⁵ Perhaps the fact that my measurements were made on dark-adapted rods accounts for this discrepancy, for in rod cells the outer member is said to become longer and slenderer in darkness than in light.

Diffused daylight

30 minutes, rods two-thirds extended

45 minutes, maximal light adaption. (Cones also light-adapted)

Darkness

15 to 20 minutes, rods, in most cases, almost completely shortened

30 minutes, maximal dark adaption. (Cones still in position of light adaption)

The quicker response of the rods in darkness than in light is noteworthy. The rod shortens in the dark, the cone in the light; since in both cases the process of shortening is more vigorous than the lengthening, it would appear that the contractility of either type of cell is the responsible factor and the relative efficiency of light and darkness is not primarily involved. In the last analysis, however, the situation may not be reducible to such simple terms. If these responses are merely the expression of the action of light and darkness on the protoplasmic myoids, why should the direction of movement of the two elements be opposed? A discussion of this phase of the problem will be found in another place.

I attempted no experimentation upon the cones of the frog. Angelucci ('90) stated that after an exposure to candle-light for 5 minutes, the cones were strongly retracted, although other experiments of his do not seem to support this conclusion. Herzog ('05) found that at medium light intensity complete light-adaption occurred in $2\frac{1}{2}$ minutes.

The most surprising discovery in this series of determinations, taken as a whole, was the length of time required to complete the adaption of the rod and cone cells in comparison with the retinal pigment. From the results of earlier workers, I had expected the positional changes of these cells to be completed in a few minutes, hence the actual values obtained were wholly unlooked for, and were only accepted after many repetitions of individual experiments.

B. EFFECT OF TEMPERATURE (NORMAL ANIMALS)

a. Retinal pigment

No investigations have hitherto been made to determine the effect of temperature on the retinal pigment of fishes, whereas several workers have used the frog for experimentation of this kind. The problem considered here was to determine the response of the retinal pigment of normal fishes and amphibians to various temperatures, both in light and in darkness.

1. *Fishes.* Experiments in the light were performed in the following way. Light-adapted⁶ fish were placed in large battery jars close to north windows where they received strong diffuse daylight; sheets of white paper were always placed under the jars to aid reflection.⁷ The highest temperature to which it is safe to subject fishes is about 25°C., although by gradual elevation a somewhat higher temperature can be withstood (Loeb and Wasteney, '12). A low temperature that did not vary beyond the limits of 3° and 5°C. was produced by introducing small pieces of ice directly into jars with the fish. At this temperature fish are for the most part inactive, the respiration rate decreases and they remain quietly at the bottom of the jars.

At the end of an experiment, which was never less than three hours long, the eyes were excised and immersed in fixing fluid in the light.⁸

During the earliest trials retinas from the same fish were compared, eyes being subjected to the extreme temperatures in

⁶ The terms 'light adaption' and 'dark adaption' as used throughout this paper imply that the animals had been previously subjected for a minimum of 4 hours either to bright diffuse daylight or to total darkness.

⁷ The possibility of a dark background influencing the distribution of retinal pigment was considered. Such a visual control, if present, would correspond to the known rôle of the eye, as determined by Pouchet ('76) and others, in animals which adapt their body color to the immediate environment. A series of careful comparisons, however, failed to show any recognizable differences between the pigment distribution in the retinas of fishes that had been kept over dark or light backgrounds.

⁸ In all work which involved the use of temperature, the fixing fluid was kept at the same temperature as that at which the experiment had been performed. Such treatment eliminated a possible source of slight error.

successive experiments. At first this seemed to be the correct procedure, but rigorous controls showed that such precautions were unnecessary. When interpreting the results of experiments conducted in the light it is not the absolute amount, but rather the relative distribution of pigment that serves as a basis for decisions. In experimentation in the dark, absolute differences in the degrees of pigmentation could give rise to errors in judgment, for the pigment, gathered into compact masses in the cell 'cups' might mask or apparently reverse the effect of temperature. Fortunately, however, the retinas, as judged from the expanded light condition seem, on the whole, to be very equally pigmented and the width of the contracted pigment zone, therefore, gives a fair index of the effect of temperature in the dark. When working in the light especially, it was found to be very desirable to have the experiments at contrasted temperatures conducted simultaneously in order that advantage might be taken of identical light conditions, for as will be shown, light intensity is an important factor in obtaining the maximum expansion of pigment.

Experimentation in the dark was conducted as follows. A fireless cooker, lined with black paper, was used as a dark chamber, on account of the minimal loss of heat incurred by it during the course of an experiment. If such an apparatus be previously brought to the temperature of the introduced jar of water, an experiment can be continued for several hours without further attention. In all determinations mentioned in this paper which were conducted in the dark, precautions were taken against the possible influence of light during the few seconds necessary for excision and transference of eyes to the fixative. A long series of careful comparisons showed identical results whether the operation was carried out in total darkness or in light of just sufficient strength to permit the operator to see the animal and his instruments. Indeed, the results obtained by operating in an ordinarily lighted room showed no recognizable differences in either pigment, rods, or cones from those secured by working in darkness.

A detailed description follows of the conditions found in each of the four fishes:

1. *Ameiurus*. At 25°C. in the light (fig. 2), the characteristic position of the expanded pigment is in a broad band about 95 μ wide, which extends nearly to the external limiting membrane. The pigment granules are evenly distributed and show no tendency to aggregate distally.

At 15°C. the condition is very similar to that just described. The pigment, on the whole, tends to be homogeneously distributed, although in many retinas at this temperature there is a slight distal accumulation.

The disposition of pigment at 5°C. is markedly different (fig. 1) for it migrates to an extreme distal situation and forms a dense zone, approximately 30 μ wide, close to the external limiting membrane, although the pigment of fishes and amphibians, under normal conditions, never actually touches this membrane. Between this heavy pigment-mass and the bases of the cells lie scattered granules, but the intervening space, nevertheless, appears relatively devoid of pigment. This extreme condition is best produced on the brightest days, and it is impossible to obtain as complete a migration on cloudy days, regardless of the temperature. On the other hand, the uniform distribution characteristic of incomplete expansion at 25°C., is independent of the intensity of diffuse daylight. This would suggest that a high temperature is more efficient than light in the regulation of pigment distribution, and that cold, that is, the absence of heat, merely allowed light to act unrestrained. Light and high temperature, then, are antagonistic in their effects.

The results at 5°, 15°, and 25°C. are, in a way, what might have been expected. A temperature of 15° to 25°C. probably represents the greatest average warmth to which the animal is subjected in nature; this range from 15° to 25°C., then, represents the limits of what may be called a warm environment for the animal. In the same way from 0° to 10°C. may be called a cold environment, and from 10° to 15°C. a neutral environment, neither particularly warm nor cold. Hence it is not surprising that the results at 15°C. are more similar to those at 25°C. than at 5°C. At 10°C. the distribution of pigment approximates rather more closely that at 5°C. A curve, therefore, obtained

by plotting temperatures as abscissas and the quantitative amount of distal migration as ordinates would show a gradual slope from 0° to 10°C., from 10° to 15°C. a rapid drop and from 15° to 25°C. a nearly horizontal but slightly sloping line.

The results of experiments performed in the dark, where the pigment is highly contracted, are usually not as clear cut as those just described. The reason for this is because it is impossible to see the qualitative distribution of the pigment granules and hence decisions regarding the effect of temperature must depend largely on measurements of the width of the narrow pigmented layer. Such a criterion, as has previously been pointed out, is open to the criticism that individual eyes may vary enough in the absolute amount of contained pigment to disconcert judgments concerning the effect of temperature. After having studied a great number of preparations, I do not believe that such an unequal pigmentation is in truth a factor that warrants serious consideration. However this may be, an obvious precaution consists in the prolonged repetition of each type of experiment. It may be said that in the course of my experimentation on the effect of temperature on normal fishes alone, over 200 retinas have been examined.

The evidence obtained from *Ameiurus*, was more conclusive than that from any of the other fishes, with the possible exception of *Carassius*. At 25°C. (fig. 4) the pigment forms a densely contracted layer, the mean width of which is about 25 μ . In contrast with this is the condition at 5°C. (fig. 3), where the cells have short pigmented processes, the total extent of which is approximately 38 μ . The differences apparent at these two extremes of temperature were so slight in comparison with the much greater variation in the light, that thorough experimentation at the intermediate temperature of 15°C. was not attempted, although it was sufficiently demonstrated that the results at this temperature do not vary to any great extent from those at the two extremes, and probably more closely approximate the highly contracted condition at 25°C.

The results of some of the temperature determinations were not conclusive. Reference has already been made to the fact that

on dull days maximal expansion in the light was hard to obtain. Among fishes in general, more doubtful cases occurred in experiments conducted in the dark than in the light, yet in all such cases the uncertainty merely lay in deciding between two nearly equal conditions, while in practically no instance was there evidence of a definite reversal whereby a greater distal migration occurred at 25°C. than at 5°C.

2. *Fundulus*. The conclusions reached from the study of *Fundulus*, as well as from the other fishes, are similar to those given for *Ameiurus*, but each fish shows individual peculiarities in the disposition of the pigment and these will be briefly described.

In the light the pigment of *Fundulus* tends to migrate to a great extent forming a broad zone at the distal ends of the cells, much denser and more sharply defined than in *Ameiurus*. Between this zone, which has a width of 30 μ , and the bases of the cells there is a clearer area, almost devoid of pigment at 5°C. (fig. 5), while at 25°C. (fig. 6) this region contains a considerable amount of evenly distributed pigment granules. In the latter case, however, the pigment still forms a closely aggregated zone distally, although it is reduced to a width of 17 μ . As in *Ameiurus* the condition at 15°C. more closely resembles that at 25°C. than at 5°C.

A peculiarity in preparations of the light-adapted retina of *Fundulus* (at least with Perenyi's fixation) is that at the higher temperatures the pigment extends in columns from base to periphery of individual cells (fig. 6), while between such columns of adjacent cells are elongated areas free of pigment but taking the plasma stain. Examination under high magnification does not show the presence of excessive shrinkage, although a casual observation might suggest that this had occurred. Where the pigment is aggregated at the base and periphery of the cells, cell boundaries are not distinguishable and the pigment appears as homogeneous masses. The separate columns connecting these two continuous zones give the whole an appearance not unlike a ladder with rungs set very close together.

In the dark the contraction is very incomplete and sometimes tends to give rise to a distal accumulation of pigment which faintly resembles the distribution in the light, although such a condition is not constantly present as a characteristic of dark adaption. It is impossible to state the cause of this pigment massing. It may be due to a greater activity at the distal ends of the cells in producing a contraction, or what would bring about a similar end result, a contraction of pigment en masse.

Measurements were taken of preparations at the extreme temperatures in the dark, the degree of variation being greater than in any of the other fishes studied. Thirteen retinas at 5°C. (fig. 7) showed the pigment to be extended a mean distance of 20.4 divisions of the ocular micrometer, whereas fifteen retinas at 25°C. (fig. 8) had a corresponding value of 14.4.

3. *Abramis*. In the light the condition in the eye of this fish is somewhat similar to that in *Fundulus*. The pigmentation is very heavy, forming a broad zone near the external limiting membrane; between this distal zone and the base of the pigment cells pigment granules are also present, the density of pigmentation depending on the temperature.

At 5°C. (fig. 9) the distal zone is wider than at 25°C. (fig. 10) in the ratio of 50 μ to 38 μ , while the proximal area is much less heavily pigmented than at 25°C. Although each area is sharply defined, the one appears to grow at the expense of the other. It was not possible to get so complete a distal accumulation of the pigment as in *Fundulus* and, as will be presently shown, the extent of migration in *Fundulus* is still less complete than that in *Carassius*.

The dark phase is one of great contraction, and although some retinas show evident differences at the extreme temperatures, yet throughout the whole set judgment of the eye has to be supplemented by actual measurements. Such measurements show that the mean expansion at 5°C. (fig. 11) exceeds that at 25°C. (fig. 12), the values being 30 μ and 20 μ respectively.

4. *Carassius*. The eye of the goldfish is more heavily pigmented than those of the three other fishes. At 5°C. in the

light (fig. 13) all the pigment is located distally forming a broad, dense zone. Between this band and the base of the cells is a narrow clear area in which scattered pigment granules are visible only with the aid of high magnifications—for practical purposes it may be said to be free of pigment.

The situation at 25°C. in the light (fig. 14) is variable. In some cases it closely simulates that at 5°C., although the clearer space is always relatively more heavily pigmented; in other instances, the pigment is uniformly distributed from the proximal to the distal extent of the cells.

The contraction that usually occurs in the dark was less pronounced in *Carassius* than in any fish heretofore described; in fact in some eyes that had been subjected to a low temperature, the actual breadth of the pigment layer nearly equalled that of a light-adapted eye. The relative expansion at the extreme temperatures in the dark, however, leaves no doubt that at 5°C. (fig. 15) the contraction is less than at 25°C. (fig. 16). Measurements in terms of the divisions of an ocular micrometer taken from eight eyes at 25°C. and ten eyes at 5°C. gave respective mean values of 8.0 and 12.1.

From observations on these four genera certain generalizations are suggested. The degree of pigmentation in the eye of *Ameiurus* (figs. 1, 2) is much less extensive than in the other fishes, as a comparison with *Carassius* (figs. 13, 14) shows in a striking manner. The three other fishes, however, offer better opportunities for comparison since their pigmentation is more nearly equal. At 5°C. in the light the pigment of both *Carassius* and *Fundulus* (figs. 13, 5) migrates distally to such an extent that a proximal zone, devoid of pigment, is created. Moreover, the dark phase of the pigment, in both these animals (figs. 16, 8), is one by no means extreme when compared with the highly compact layer in *Abramis* and *Ameiurus* (figs. 12, 4). In the last named fishes, on the contrary, it is impossible under the most favorable conditions of light and temperature to obtain the proximal clearer area entirely free from pigment; correlated with this absence of complete expansion is the high degree of contraction which is evoked in the dark.

It does not seem probable that quantitative differences in the degree of pigmentation can be the cause of such relations, for if the relative amounts and the distribution of pigment in *Fundulus* and *Abramis* be compared at 5°C. in the light (figs. 5, 1) and at either temperature in the dark (figs. 7, 3) it must be admitted that quantitative differences do not adequately explain the conditions that exist.

A clearer insight is gained if these responses are viewed from the standpoint of cell organization. We can think of two general types of pigment cells in which the pigment distribution is correlated with the behavior outlined above. Thus, in one type, the pigment would tend to remain at the distal end; such a cell would show maximal expansion but relatively incomplete contraction. In a second type of cell, in which the pigment aggregates more proximally, maximal contraction but incomplete expansion would be accomplished. Although other species of fishes should be studied before a final conclusion is reached, this set of relations may be general. The correlation can be stated, at least tentatively, as follows—the highest degrees of expansion and contraction in the retinal pigment of fishes are mutually exclusive in the same retina.

2. *Frog (adult and larva)*. As in many other lines of physiological work, the frog has been used to a large extent by investigators of retinal physiology.

Ewald und Kühne ('78) performed the first experiment in which the position of the retinal pigment of the frog was shown to be dependent upon temperature. According to their account, after 2 hours' immersion in ice water in the dark, the pigment showed a distribution similar to that obtained at 17°C., which may be called a state of contraction.⁹ When, however, frogs were subjected to 30°C. for 2 hours, an expansion occurred in which lightly pigmented processes were said to extend even to

⁹ This, however, does not coincide with an earlier statement ('77, p. 250) of the same authors, "Vor allem ist die Temperatur von ausserordentlichem Einflusse Frösche, welche 1-2 Stunden in Eiswasser gehalten wurden, liefern schwarze Netzhäute, indem das ganze Epithel mit ausschläpft, und nicht viel besser verhalten sich die Präparate von solchen, die bei 5°-10°C. im Dunkeln verweilen."

the external limiting membrane. These workers were primarily interested in discovering under what conditions of temperature an accumulation of pigment in the rod area could be avoided, for their investigation dealt with the visual purple. Since the frogs used for these determinations were treated with curare in order to produce pigment relaxation, and, moreover, since these animals were in an oedematous condition as a result of this poisoning, it is evident, as Herzog ('05) pointed out, that much weight can not be given to their conclusions alone.

To Gradenigro ('85) belongs the credit of having performed the first temperature experiment upon the retinal pigment of normal animals. He introduced a dark-adapted frog into a dry, darkened chamber and removed the whole to a dark room. A temperature of 30°C. was maintained until heat rigor set in, when, on examination, the pigment was found to be in a condition of maximal light expansion (fig. 19). Gradenigro's results were confirmed by Angelucci ('90) and by Fujita ('11).

Herzog ('05), without knowledge of Gradenigro's contribution, undertook a detailed study of the relation between temperature and pigment distribution. His results not only corroborated those of Gradenigro, but also established the additional facts that at low temperatures (0°-14°C.) in the dark the distribution of pigment is identical with that at high temperatures (fig. 17), while only between 14° and 18°C. in the dark (fig. 18) is maximal pigment contraction obtained.

His experiments were performed in the following manner. Dark-adapted frogs (*R. temporaria* and *R. esculenta*?) were placed in a heating chamber at an initial temperature of 20°C., the introduction of the animals cooling the apparatus to 17°-18°C. Progressive heating raised the temperature in 15 minutes to 24°C., in 30 minutes to 32°C., in 45 minutes to 37°C., and in one hour to 39-40°C. At each fifteen minute interval frogs were removed and their eyes prepared for microscopical examination.

At 24°C. the position of the pigment was not essentially different from the normal state of maximal contraction, although delicate fringed processes did extend towards the external limiting membrane. It is probable that such an experiment did

not fairly test the effect of this temperature on pigment migration. One could hardly expect the body temperature of the animals to become adjusted to that of the apparatus in such a short time, especially since the heating was progressive and the final temperature was not realized until the end of the experiment.

At 32°C. the pigment had extended to the maximal distance but the distribution was nearly homogeneous.

A condition of extreme expansion occurred at 37°C. A dense massing of pigment near the external limiting membrane masked the rod ellipsoids completely, while the outer members of the rods were nearly free from pigment. When the temperature was raised to 39° or 40°C. clonic spasms occurred which ended in death; the pigment, nevertheless, retained the same position as at 37°C.

A second series of experiments, in which frogs were cooled in a refrigerating apparatus, showed that a subjection to 0°C. in the dark for 2 hours produced incomplete expansion, while after 3 hours the pigment was distributed in a zone of maximum breadth, but with only a slight tendency toward distal massing. This discovery, which was Herzog's most interesting contribution, is not only in disagreement with the commonly quoted result of Ewald und Kühne's earlier work, but also has not been substantiated by the recent investigation of Fujita ('11), who, however, states that high temperature does induce pigment expansion, as the other investigators have all maintained. Fujita tried the effect of low temperature on only four animals, the duration of his experiments ranging from 30 minutes to 6 hours, yet he drew the following positive conclusion (p. 170): "*Das Resultat war in allen Fällen das gleiche: ich konnte keine Hellstellung konstatieren. Die Zapfen waren nicht kontrahiert, das Pigment nicht vorgewandert.*"

Since all these results on the frog's retinal pigment are not only fundamentally different from those found by me in fishes, but also have no parallel in the movements of vertebrate and invertebrate melanophores, and since, moreover, there is no general agreement concerning the effect of low temperature, a thorough reinvestigation of the problem seemed to be needed.

My first effort, therefore, was to repeat Herzog's work using apparatus and methods that essentially agreed with his, in order to ascertain whether identical results would be realized. If the movements of the frog's retinal pigment are really exceptional among other lower vertebrates, such responses have considerable theoretical as well as incidental interest.

The following apparatus was devised. A cubical cage, made of fine wire netting with a cover of the same material, was supported by uprights inside a large battery jar which was fitted with a glass cover perforated by a small hole to allow slow diffusion of air. The wire cage, made to receive the frog, did not come in contact with any part of the surrounding glass receiver which was to serve as a constant temperature chamber. The glass receiver sat upon a platform in a large cylindrical metal tank. A thermometer passed through the cover of the tank and also through the cover of the temperature chamber into that chamber itself. A funnel connected with a rubber tube to exclude light was fitted into the cover of the tank and served for introducing water into the tank.

Tests were conducted in the following way. A dark-adapted frog was wiped dry and placed within the wire cage¹⁰ inside the constant temperature chamber. Previously the chamber had been brought to the desired temperature by one of two simple methods. If the effect of heating was to be studied, the metal tank was partially filled with water at the appropriate temperature and the whole system allowed to adjust itself until the thermometer inside the constant temperature chamber registered the required degree of warmth. After the frog had been introduced, the temperature could be regulated without opening the apparatus by admitting warmer water through the funnel and drawing off an equivalent amount from the bottom of the tank. If, on the other hand, a temperature near the freezing

¹⁰ Herzog lays much stress on the facts that the animal's body was never in contact with solids other than the wires of the cage, and that the body was always wiped free from secretions or excretions at the commencement of an experiment. He seems to fear lest there should be a chemical stimulation due to heated secretions or excretions that would affect the results in case these precautions were not followed.

point was desired, the outer chamber of the tank was filled with a mixture of ice and water; if a sufficient amount of ice was provided a temperature of from 3° to 5°C. could be maintained, without further attention, throughout the whole experiment. At the expiration of the time allowed for temperature adaption (usually 3 hours), the apparatus was placed in the dark or in weak red light and the frog's eyes were removed and fixed in darkness at the same temperature as that at which the experiment had been conducted.

In the course of these tests nearly 100 eyes were examined, yet the general results obtained were identical with those described by Herzog. At 3°C. and 33°C. (figs. 17, 19) the pigment was expanded approximating the condition characteristic of light; between the temperatures of 14°C. and 19°C. (fig. 18), however, the pigment was contracted to a narrow compact layer. It should be noted that 14°C. and 19°C. may not represent the limiting temperature at which pigment contraction occurs, although Herzog states this to be the case; no attempt was made by me to determine these intermediate temperature-limits. At low temperatures the expansion of pigment was generally not as complete as at a high temperature or in the light, and there was often considerable variation in different parts of the same retina, yet the general result was one of unmistakable expansion.

In some cases, however, there was little or no evidence of a pigment expansion at the lower temperature, yet such instances were comparatively rare. Although the cause of discrepant results of this kind is not evident, they perhaps furnish additional proof for the nervous control of the frog's retinal pigment, as many workers maintain (*vide infra*). It seems probable that Fujita was unfortunate enough, in the few experiments which he performed at a low temperature, to obtain nothing but this lack of typical results, although in my own work the occurrence of such anomalous cases was always sporadic.

A careful comparison was made of the results obtained at 3°C. and 33°C. No constant difference in the amount of migration could be detected, although Herzog states that at 37°C. the distal migration is greater than that obtained at low temperature.

Hence we may conclude that the condition found in the frog is unlike that found in the fishes. It should be noted, however, that between certain limits the two animals show similar tendencies in their pigment responses. These limits are approximately 0° to $19^{\circ}\text{C}.$ in the dark for the frog, and 0° to $28^{\circ}\text{C}.$ in either darkness or light, for the fishes.

Since the tendency of pigment migration under the influence of temperature agrees between the limits of 0° to $19^{\circ}\text{C}.$ for the dark adapted frog and 0° to $28^{\circ}\text{C}.$ for the fishes either in light or in darkness, the query may be raised—is it not possible that if the fishes were subjected to higher temperatures a reversal of the temperature effect would occur in which an expansion of the pigment would again be found as in the frog? The fact that Herzog found but slight differences at $18^{\circ}\text{C}.$ and $24^{\circ}\text{C}.$ increases this suspicion. I am convinced, however, that a tendency toward such a response does not exist in the retinal pigment of fishes even to the slightest extent. In the first place, as stated before, prolonged heating of the frog's retina at $24^{\circ}\text{C}.$ would be likely to produce more striking changes than Herzog obtained. Furthermore, a few experiments in which the temperature of fishes was raised to $30^{\circ}\text{C}.$ and over failed to show, in either light or darkness, anything beyond the characteristic response of less complete expansion than at the lower temperature.

The extent to which the retinal pigment of the frog and of fishes moves under the influence of temperature differs in a high degree. Among the fishes the differences are small and amount to little more than a redistribution of pigment in the dark and light phases respectively, whereas in the dark-adapted frog varying temperature induces the whole range of pigment response usually occasioned by light or darkness. This further indicates that the nature of the response in the two kinds of pigment cells differs fundamentally. In the fishes probably the response is through the direct action of temperature on the cell protoplasm, while in the frog the pigment migration may be produced indirectly through the intervention of the nervous system.

All the experiments of previous workers on the effect of temperature have been performed in the dark and I, therefore, set about to discover what results would be obtained in the light. The only change in the apparatus from that previously described was that a 20 litre jar replaced the metal tank; this, when filled with water at the desired temperature, performed the necessary heating function while its transparency did not interfere with the entrance of light into the inner chamber. Experiments were made at the same temperatures as in the dark— 3° to $5^{\circ}\text{C}.$, $16^{\circ}\text{C}.$ and $33^{\circ}\text{C}.$, but the results were, for the most part, conditions of uniform expansion independent of temperature.

It was observed that when frogs were subjected to a low temperature they became quiescent and tended to keep their eyelids closed. Although the lower lid (the only one which is movable to any extent) is more or less transparent, the possibility of its influencing the results led to its removal in a number of instances; no difference, however, was obtained by the observance of this precaution. From these results, therefore, we conclude that in darkness, temperature is the controlling factor, while in the light temperature is subordinate to the stronger stimulus, light.

At this juncture a doubt arose as to the exact temperature the frog's retina was experiencing while in the apparatus. It is well known that at the surface of the frog's body rapid evaporation can take place; hence it is perfectly conceivable that the rate of evaporation in the temperature chamber might be such as to keep the body temperature for some time considerably below that of the surrounding air. This possibility was first checked by taking the oesophageal and rectal temperatures of animals that had been subjected to various temperatures in the apparatus for several hours. The recorded temperature, however, was never found to vary more than a fraction of a degree from that of the air in the containing chamber.

To be absolutely certain on this point, a prolonged set of experiments was made in which the frogs were immersed in water media of appropriate temperatures. It is certain that after a short time the animal, as a whole, must assume the temperature

of the medium irrespective of activities at the surface of the body.

The apparatus for this verification consisted merely of a large battery jar, a sheet of coarse wire gauze and suitable weights. The jars were filled with water to within a quarter of a centimeter of the top, and the gauze, held in place by weights, served as a cover. This device worked in the following manner. Animals coming to the surface to breathe could only get their nostrils above water, the rest of the head and body remaining submerged, hence, in a short time the body temperature of the frog necessarily approximated that of the surrounding medium.

At 3°C. the body temperature of the animal quickly fell, the respiration rate was reduced until it practically ceased and the bodily activities diminished until the frogs, for the most part, remained quietly at the bottom of the jar, although some animals would occasionally swim to the surface to breathe. At 33°C., on the contrary, the frogs were very active and had to return at short intervals to the surface, where they would sometimes remain for several minutes clinging to the netting.

A series of experiments was performed both in darkness and in light. In the dark nothing new was learned beyond the conditions already described. In the light, the first experiment showed a state of extreme pigment expansion at 3°C., which was comparable to that of *Ameiurus* under similar conditions. The other trials, at 16°C. and 33°C., on the contrary, showed the pigment uniformly distributed. Another experiment, a short time after, gave the same result at 3°C. but not so decisively. The possibility of discovering a similarity in the pigment responses of frogs and fishes in the light, led me to repeat these experiments many times without, however, again obtaining similar results.

If extreme pigment expansion occurred at 3°C. it would be interesting from another standpoint since Herzog reported a similar condition, in darkness, at the highest temperatures which these animals can withstand.

Occasionally during experiments both in light and in darkness, an anomalous condition arose whereby the distribution

of pigment in one part of the retina was markedly different from that in the remaining portions.¹¹ Such conditions may have been due to a variety of disturbing factors, Angelucci ('90) has recorded noises, unilateral pressure on the eyeball and mechanical or electrical stimulation of the body as causing the migration of pigment in dark-adapted animals. Herzog ('05) likewise states that a frog tied up for 24 hours in the dark showed the pigment in the light position. A whole series of experiments and observations are on record to show motor control of some sort not well understood. It certainly is evident from a comparative study of pigment in other forms, that is, in the retinas of fishes as well as in vertebrate and invertebrate melanophores, that the situation in the frog is entirely unlike that in any other animal concerning which we have data.

Herzog explained the temperature responses of the frog's pigment in the following way. If the effect of temperature is purely physical, its action presumably consists in accelerating or retarding chemical processes in the protoplasm of the pigment cell. Since, however, the movements of the dark-adapted pigment are not directly correlated with the temperature gradient, a physical action of temperature is probably not responsible for the observed phenomena. If, on the other hand, it is assumed that the response of the pigment involves the principle of 'specific energies,' then any positive stimulus, acting through the nervous system, will cause a pigment expansion, and thus a satisfactory explanation for the known facts is furnished.

In connection with the special case offered by the frog an interesting speculation arises as to the kind of pigment responses shown by the frog larva. The larva, in a general way, is comparable to a fish; at least it may be said that the larval stage recapitulates certain conditions persistent in the adult fish. Is it not possible, therefore, that under the action of temperature the pigment of the larval frog will show a distribution similar to

¹¹ A somewhat similar lack of consistency was also noted by Fick ('90), in his attempts to obtain maximal contraction in dark-adapted eyes. He concluded, however, that inequality in the pigment distribution was characteristic of dark-adapted retinas.

that in fishes? The material, on which an answer to this question was sought, was the larva of the bullfrog (*R. catesbiana*). Animals were obtained during the month of April, 1914; these, of course, represented larvae hibernated from the previous season, since two or even three years are required to complete the metamorphosis of this species. A few experiments were also made on animals obtained in November, 1914.

From the larvae procured, two size limits were selected for experimentation. The smallest larvae had a total body length of 4.5 cm., the hind legs of such animals not being visible; the largest larvae had a body length of 7.0 cm., and the hind legs were developed as two small buds with the digits just differentiated.

In neither the 4.5 cm., nor the 7.0 cm. animals were the eyes as deeply pigmented as in the adult of *R. pipiens*. Of the two sizes, the 7.0 cm. larvae had the pigment more highly developed, and consequently better differentiation was obtained at the various temperatures with these, than with the smaller animals.

The 4.5 cm. larvae at 3°, 26°, and 32°C. in the dark showed the pigment in an expanded state (cf. figs. 20, 22) but not in a firm zone of uniformly distributed granules. On the contrary, the cells displayed pigmented processes that seemed to be more or less independent of each other; the appearance of the zone being that of a more uniform base with a fringe of pigment extending distally from it. The degree of expansion at 26°C. was distinctly less than at either 3°C. or 32°C., although at neither of the extreme temperatures was the pigment as fully extended as in the light. At 16°C., however, a striking difference was found (cf. fig. 21), for the pigment lay contracted in a narrow compact layer near the choroid.

The 7.0 cm. animals gave results (figs. 20, 21, 22) quite similar to those just described, although the contrasts at the various temperatures were considerably sharper due to the heavier pigmentation of the eyes at this stage.

In these larvae, therefore, the behavior of the retinal pigment to temperature is identical with that characteristic of adult frogs. Since these animals were always immersed in water, there is no

question concerning the correspondence of their body temperature and that of the surrounding medium.

What would be discovered in a study of earlier stages I can not say, but I suspect great difficulty would be encountered in interpreting the results due to incomplete pigmentation, for the differences at various temperatures in the 4.5 cm. tadpole, although fairly well marked, showed much less contrast than those exhibited by the 7.0 cm. animals.

3. *Necturus*. A comparison between the frog and some urodele suggested another interesting problem. Is the condition exhibited in the frog restricted to anurans or is it common to the whole group of amphibians? This query becomes all the more pertinent when it is recalled that urodeles are not in the direct line of ascent to the anurans; that is, they are not amphibians which have never gone beyond the water inhabiting stage, but are more probably a group that were once land animals and have again returned to the water as a secondary adaption.

The common mud puppy, *Necturus maculatus*, was chosen because of the ease with which it is procured and kept in captivity. These animals were treated according to the technique used for fishes, hence a temperature higher than 28°C. was not attempted.

The first experiments performed were to secure typical light- and dark-adapted retinas in order that some basis of comparison might be had. The results were by no means as striking as one might wish. In both cases the pigment was extended, not in a band with even contours, but generally in large conical processes from the individual cells, which, like other cells of *Necturus*, appear to be very large; these conical processes surround the distal ends of the outer segments of the rods.

In the light, the pigment was usually somewhat more extended than in darkness. The processes were not of uniform length but mean measurements may be expressed by the values of about 38 μ in the light and 30 μ in the dark,—a condition of slight contrast when compared with fishes or the frog. The presence of a certain amount of migration in *Necturus* has been previously noted by Howard ('08), who took advantage of

the contraction in the dark in his study of visual cells. This situation is comparable to that found in Triton where movements of the pigment through the influence of light were discovered by Angelucci ('78), Stort ('87) and Garten ('07). The extent of pigment migration in this animal is described as being very limited by Garten (p. 70) who says: ". . . dieselbe ist aber hier unvergleichlich viel schwächer als bei vielen anderen niederen Wirbelthieren."

In an examination of the effects of various temperatures on dark-adapted eyes, however, no constant differences were noted. In some instances the processes of the cells seemed to be less heavily pigmented and the bases more heavily pigmented at 15°C. than at the two extreme temperatures, but this was by no means constant. At least, it can be said that there is no marked contraction of the pigment in the dark at any temperature.

The evidence from *Necturus* and the limited pigment migration in Triton conclusively prove that the pigment responses typical of the frog are not common to all amphibians. It is probable, therefore, that such peculiarities as were described for the frog have been developed solely within the anuran group.

b. Visual cells

Although the myoids of both the rod and cone cells of fishes are capable of a high degree of contractility (a 90 per cent retraction occurs in some instances), the effect of various temperatures on these cells remains untried up to the present time; in fact, the frog is the only animal upon which such work has been attempted. Gradenigro ('85), Angelucci ('84b), Herzog ('05), and Fujita ('11) found that warming produces the same effect on the cones of the frog as does light. Herzog also stated that cooling to 0°C. likewise caused the cones to shorten, although Fujita denies that this occurs.

There is no record of any attempt to determine the effect of temperature upon the rods of vertebrates beyond the statement of Gradenigro ('85) that at 30°C. the rod of the frog shortens as in light.

The object of the work upon visual cells to be described in this section parallels that stated for pigment, that is, to determine the effects of various temperatures on the myoid of rod cells and of cone cells in normal fishes and amphibians.

The apparatus and technique employed were similar to those which were used in the experimentation upon pigment. Particular care was exercised at the termination of experiments conducted in the dark to guard against the action of light on the highly sensitive visual cells. In the fishes, measurements of the cone myoid were made from the external limiting membrane to the proximal edge of the ellipsoid, and in the frog, from the external limiting membrane to the proximal side of the oil drop which is situated at the distal end of the ellipsoid. The lengths of the rods were measured from the junction of the inner and outer members to the external limiting membrane. Each value given in the tables represents the mean measurement, in micra, of many (12 to 24) individual elements.

1. *Fishes*. (1) *Ameiurus*. Of the four fishes studied, the cones of *Ameiurus*, in many ways, gave the least satisfactory results. These elements are not located at uniform levels and the differences between the elongated and shortened condition, when stimulated by extremes of temperature, are not striking to the eye. The additional fact that the cones, when maximally shortened under the influence of temperature, never closely approach the external limiting membrane makes these animals rather unsatisfactory for certain kinds of experimental work.

Tables 1 and 2 present data for both rods and cones from typical retinas at 5°C. and 25°C. in the dark.

It will be seen that at the higher temperature the myoids of both cones and rods lengthen (figs. 32, 33). Especially in the cones is this response unmistakable. The lengths of the rod's inner member, after subjection to the extreme temperatures, varies within only a few micra, yet the relative change may be 25 per cent or more; moreover, since the mean ranges at the extreme temperatures do not overlap, these differences are presumably significant. If the length of the rod ellipsoid, 4 μ ,

TABLE 1

Measurements from the retinas of four Ameiurus which had been kept at 5°C. in the dark. The values are in micra and represent measurements taken along axes coinciding with radii of the eyeball

NUMBER OF ANIMAL	NERVE FIBER LAYER TO EXTERNAL LIMITING MEMBRANE	CHOROID TO EXTERNAL LIMITING MEMBRANE	CONE MYOID	ROD INNER MEMBER
1	35	95	19-32	7-9
2	35	95	13-20	7-9
3	50	95	19-31	9
4	43	100	13-24	9-10
Mean.....	41	96	16-27	8-9

TABLE 2

Measurements from the retinas of four Ameiurus which had been kept at 25°C. in the dark. The values are in micra and represent measurements taken along axes coinciding with radii of the eyeball

NUMBER OF ANIMAL	NERVE FIBER LAYER TO EXTERNAL LIMITING MEMBRANE	CHOROID TO EXTERNAL LIMITING MEMBRANE	CONE MYOID	ROD INNER MEMBER
1	50	105	32-38	9-13
2	43	100	32-38	12-15
3	42	87	25-31	10
4	50	93	31-42	9-12
Mean.....	46	96	30-37	10-13

is subtracted from the mean values, the relation existing between the corresponding values at either end of the temperature range will be expressed by the following proportion: 25°C.: 5°C. = 3:2. The variability in the length of adjacent rods in any section is relatively so great that this disparity in the length of the myoids is not apparent until actual measurements are made.

In a few observations upon the effect of temperature on the extended rods in the light, the range at 25°C. seemed to run higher than at 5°C. by 15 to 20 per cent. The lengths of the extended inner members were approximately 50 μ at 5°C. and 60 μ at 25°C. Similar tendencies will be noted among certain other fishes.

(2) *Abramis*. The cones, with their large oval ellipsoids $10\ \mu$ in length, are very conspicuous in stained preparations. The fine rods, on the contrary, are not always demonstrable when treated with the Ehrlich-Biondi stain, which acts in an unusually capricious fashion in respect to these elements.

The elongated cone cells are more or less uniformly extended, although the variability in length is greater under these conditions than when in the retracted state. The rods, however, are arranged in the dark at very irregular levels so that retinas present a fairly even distribution of them from $8\ \mu$ to sometimes as far as $50\ \mu$ from the external limiting membrane. In the light the condition is one of more uniform elongation.

In tables 3, 4, and 5, are presented the data obtained from measurements at 5° , 15° , and 25°C . in the dark.

TABLE 3

Measurements from the retinas of four Abramis which had been kept at 5°C . in the dark; the values are in micra and represent measurements taken along axes coinciding with radii of the eyeball

NUMBER OF ANIMAL	NERVE FIBER LAYER TO EXTERNAL LIMITING MEMBRANE	CHOROID TO EXTERNAL LIMITING MEMBRANE	CONE MYOID	ROD INNER MEMBER (MAXIMUM)
1	90	90	7-12	40
2	72	85	5-12	
3	85	91	5-9	
4	82	88	6-10	
Mean.....	82	89	6-11	40

TABLE 4

Measurements from the retinas of three Abramis which had been kept at 15°C . in the dark; the values are in micra and represent measurements taken along axes coinciding with radii of the eyeball

NUMBER OF ANIMAL	NERVE FIBER LAYER TO EXTERNAL LIMITING MEMBRANE	CHOROID TO EXTERNAL LIMITING MEMBRANE	CONE MYOID	ROD INNER MEMBER (MAXIMUM)
1	95	94	16-29	59
2	100	105	15-24	60
3	88	69	24-34	25
Mean.....	94	89	18-29	48

TABLE 5

Measurements from retinas of four Abramis which had been kept at 25°C. in the dark; the values are in micra and represent measurements taken along axes coinciding with radii of the eyeball

NUMBER OF ANIMAL	NERVE FIBER LAYER TO EXTERNAL LIMITING MEMBRANE	CHOROID TO EXTERNAL LIMITING MEMBRANE	CONE MYOID	ROD INNER MEMBER (MAXIMUM)
1	80	89	21-49	44
2	72	84	28-40	
3	105	84	30-40	
4	76	65	28-40	44
Mean.....	83	81	27-42	44

The effect of temperature, therefore, upon the cones of Abramis is very marked, the length of the myoid at 5°C. (fig. 25) averaging only 25 per cent of that at 25°C. (fig. 27), while in extreme cases this ratio is as low as 10 per cent. If the mean limits of myoid extension are averaged, values of 9, 24, and 35 micra are obtained for the temperatures of 5°, 15° and 25°C. respectively. These values are in the ratio of 1.0: 2.5: 4.0, or in other words, the 'coefficient of expansion' for the myoid of Abramis is $2 \pm$ for 10°C.

As a matter of fact, in the great majority of these temperature experiments, 5°C. represents a value too high and similarly 25°C. a value too low for the actual temperatures maintained. 3°C. and 26°C. are more nearly the actual values. If temperature is plotted as abscissas and the myoid length in micra as ordinates, the resulting curve (fig. A) is a straight line showing that the temperature effect is uniform between these limits.

The straight line obtained in the plot may indicate that the temperature response is the result of two or more opposed chemical reactions which operate in a compensatory manner. Since the response of the myoid in elongating is directly correlated with the temperature gradient, it seems feasible that the effect of temperature is physical (in the sense of Herzog), and through its action chemical processes in the protoplasm are uniformly accelerated. If the length of the myoid is a fair index of the chemical activity that causes elongation, and if the effect of

temperature is purely physical, the coefficient of $2 \pm$ for $10^\circ\text{C}.$ is of interest, on account of its agreement with the value found for the temperature coefficient of various vital processes as well as of ordinary chemical reactions.

The incomplete data concerning the maximum lengths of the inner members of rods are hardly significant, although both of the mean values at $15^\circ\text{C}.$ and $25^\circ\text{C}.$ are slightly above the one measurement at $5^\circ\text{C}.$ The Ehrlich-Biondi stain was used on most of these preparations and it was only rarely that the rod ellipsoid took the stain sufficiently to render its identification certain.

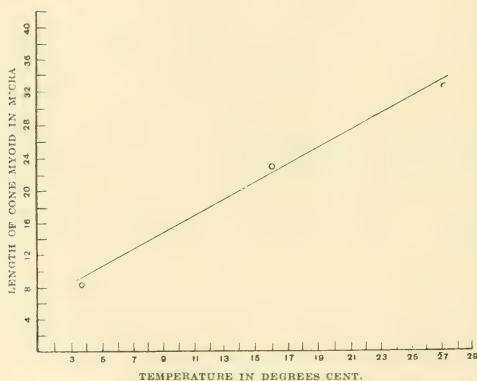


Fig. A. Plot showing the relation between temperature and myoid length in the cone cells of *Abramis*.

Table 6 gives measurements from two retinas which had been subjected to extreme temperatures in the light. The measurements for the cone myoids are identical, and in no one of the four fishes was there a demonstrable change ascribable to temperature under these conditions. It should be noted that the cone measurements at $5^\circ\text{C}.$ in the dark (table 3) are either equal to or, as in this case, are actually smaller than those representing the highly retracted light condition. This dependence upon temperature was taken advantage of in all experimentation to be described later where elongation of the cones was

desired. Although the disparity between the rod lengths given in the table is probably extreme, such values have the same purport as the corresponding measurements made on the rods of *Ameiurus*.

TABLE 6

Measurements from the retinas of two Abramis, one of which had been kept at 5°C., the other at 25°C. in the light; the values are in micra and represent measurements taken along axes coinciding with radii of the eyeball

NUMBER OF ANIMAL	TEMPERATURE °C.	NERVE FIBER LAYER TO EXTERNAL LIMITING MEMBRANE	CHOROID TO EXTERNAL LIMITING MEMBRANE	CONE MYOID	ROD INNER MEMBER (MAXIMUM)
1	5	54	78	8-14	50
2	25	88	100	8-14	75

(3) *Fundulus*. The retina of this fish is interesting because of the presence of prominent 'double cones.' Such elements are found in representatives of all the vertebrate classes, with the exception of mammals (Greeff, '00). They consist of two cones with fused inner members, although close examination is necessary to demonstrate this union. One component is usually larger and is known as the 'chief cone' (fig. 29; *chl. con.*), whereas the smaller is the 'accessory cone' (fig. 29; *con. acc.*) The chief cone alters its position independently of its accessory cone, which remains close to the external limiting membrane and is not moved to any great extent by the action of light or other stimulating agents.

The rods, although rather small, are quite in evidence and differ from those of *Abramis* in maintaining fairly uniform degrees of elongation.

In the tables (7 and 8) which show the characteristic results of experimentation in the dark, the mean myoid length of the chief cones at 5°C. (fig. 28) is only about one-third that at the higher temperature (fig. 29). The differences in the accessory cones are not striking, although the lower values are somewhat increased at 25°C. Since the movements of the accessory cones are very limited, this probably represents a significant elongation.

The contrast between the extension of the rod at 5° and 25°C. is striking and, added to the evidence gained from other fishes,

TABLE 7

Measurements from the retinas of five Fundulus which had been kept at 5°C. in the dark; the values are in micra and represent measurements taken along axes coinciding with radii of the eyeball

NUMBER OF ANIMAL	NERVE FIBER LAYER TO EXTERNAL LIMITING MEMBRANE	CHOROID TO EXTERNAL LIMITING MEMBRANE	CHIEF CONE MYOID	ACCESSORY CONE MYOID	ROD INNER MEMBER
1	100	69	5-11	1-5	16-19
2	106	68	5	1-5	
3	88	69	5	1-5	
4	105	68	3	1-4	11-19
5	75	52	5-9	1-5	16-23
Mean.....	95	65	5-7	1-5	14-20

TABLE 8

Measurements from the retinas of five Fundulus which had been kept at 25°C. in the dark; the values are in micra and represent measurements taken along axes coinciding with radii of the eyeball

NUMBER OF ANIMAL	NERVE FIBRE LAYER TO EXTERNAL LIMITING MEMBRANE	CHOROID TO EXTERNAL LIMITING MEMBRANE	CHIEF CONE MYOID	ACCESSORY CONE MYOID	ROD INNER MEMBER
1	120	87	14-22	2-5	24
2	120	87	15-22	2-6	31
3	130	100	17-26	6	21
4	135	94	17-27	6	19
5	135	110	17-26	1-3	38
Mean.....	128	96	16-25	3-5	27

indicates that in these animals a lengthening of the inner members is favored by a high temperature.

A series of measurements of chief cones in the light failed to show any differences at the extreme temperatures.

(4) *Carassius*. The retina of *Carassius*, as well as that of *Fundulus*, has prominent double cones.

In the few eyes measured, the chief cone elongated with increased temperature (figs. 23, 24) but the accessory cone did not change its position, at least to any extent.

Table 9 summarizes the results from typical retinas.

TABLE 9

Measurements from the retinas of four *Carassius*, two of which had been kept at 5°C., and two at 25°C. in the dark; the values are in micra and represent measurements taken along axes coinciding with radii of the eyeball

NUMBER OF ANIMAL	TEMPERATURE °C.	NERVE FIBER LAYER TO EXTERNAL LIMITING MEMBRANE	CHOROID TO EXTERNAL LIMITING MEMBRANE	CHIEF CONE MYOID	ACCESSORY CONE MYOID
1	25	75	88	21	3-5
2	25	69	69	21	2-3
3	5	56	63	1-3	2
4	5	69	94	3-4	2-3

2. *Frog*. (1) *Rana pipiens* (adult). Gradenigro ('85), Angelucci ('90), Herzog ('05) and Fujita ('11) have all stated that at a temperature of 30°C. or more, the cones of the frog shorten until they assume the position characteristic of light. Thus, Herzog (p. 419) says: "Aufenthalt im Brutschrank 1 1/2 Stunde lang, Temperatur von 21° bis 30° C. ansteigend: . . . dieselbe zeigt auch, dass die Zapfen maximal contrahiert sind. Die Länge der Zapfen beträgt mit ganz vereinzelt Ausnahmen 0.0091 mm. (v. d. Lim. extern.—Oelkugel excl.)."

The action of low temperature was first tried by Herzog ('05), who makes the following statement (p. 424) concerning the result of two hours' cooling to 0°C. in the dark: "Dagegen sind die Zapfen bereits nahezu höchstgradig verkürzt. Ihre Länge beträgt fast durchweg 0.0078 bis 0.0091 mm."

Fujita experimented upon six frogs, which were kept at a low temperature in an 'Eisgefäß' for periods of 30 minutes to 6 hours, after which the decapitated heads were fixed in ice-cold fluid. His conclusion (p. 170) is diametrically opposed to that of Herzog. "Das Resultat war in allen Fällen das gleiche: ich konnte keine Hellstellung konstatieren. Die Zapfen waren nicht kontrahiert. . . ."

Measurements showing the elongation of the cone myoid at intermediate temperatures (14° to 19°C.) are not given by Herzog, who, however, states (p. 418) concerning retinas that had been raised in the course of 15 minutes from 18° to 24°C.: "Ein wesentlicher Einfluss ist nicht zu erkennen. . . . Die

Zapfenlänge (von der Limitans externa bis zur Oelkugel im Ellipsoid exclusive) beträgt im Durchschnitt maximal 0.034 mm., im Minimum 0.0169 mm."

With apparatus and methods similar to those described in connection with the experimentation upon frog's retinal pigment, an attempt was made to discover the exact responses of the cone myoid to high, medium, and especially to low temperatures.

In table 10, which gives the measurements of visual cells from a few typical preparations, it will be observed that the cones are greatly shortened at 33°C. (fig. 36), but that at the other temperatures they retain the elongated condition typical of darkness (figs. 34, 35).¹² Measurements of the cones gave two modal lengths, which are approximately expressed by the figures in the table representing the extreme values. The maintenance of elongation at a low temperature is in agreement with Fujita's ('11) experiments,¹³ but is opposed to Herzog's conclusion, which was apparently based upon exhaustive investigation. It is true that the temperature of 0°C. which the latter worker used was a few degrees less than the lower limit (3° to 5°C.) employed

TABLE 10

Measurements from the retinas of eight Rana pipiens which had been kept at 3°, 14°, 19°, and 33°C., respectively, in the dark; the values are in micra and represent measurements taken along axes coinciding with radii of the eyeball

NUMBER OF ANIMAL	TEMPERATURE °C.	NERVE FIBER LAYER TO EXTERNAL LIMITING MEMBRANE	CHOROID TO EXTERNAL LIMITING MEMBRANE	CONE INNER MEMBER	ROD INNER MEMBER
1	3	140	66	12-20	10±
2	3	160	75	13-22	9±
3	14	103	56	11-20	11±
4	14	88	50	11-16	10±
5	19	103	61	13-19	13±
6	19	94	63	14-23	10±
7	33	163	63	8-12	10±
8	33	126	63	7-10	11±

¹² The actual values at medium and high temperatures vary somewhat from those given by Herzog for *R. temporaria* (and *R. esculenta*?).

¹³ This experimentation upon the retinal elements of the frog was practically completed before Fujita's paper was known to me.

by me, yet it seems improbable that such a small difference would cause the cones to shorten maximally. Any error that could be introduced during these determinations would tend to shorten the cones, hence Herzog's results are at a disadvantage in this respect. I have continued experiments for 6 hours, yet the results were always the same; in no case was there found a general shortening of the cones that in any way resembled the extreme condition at 33°C.

From these results, which, in a general way, are the reverse of those found in fishes, it is evident that the responses of the cones are not comparable to those of the retinal pigment. The pigment may indeed be under nervous control, so that stimulating agents such as heat, cold and light produce a migration according to the principle of specific energies, yet if the cone cells are influenced by the nervous system, these experiments can not be said to furnish proof of such a relation.

In order that there should be no doubt concerning the effect of low temperature upon the cone myoid, a further determination was made long after the results which are tabulated above were obtained. In this later experiment rigorous precautions were observed to eliminate possible errors. After frogs (*R. pipiens* of various sizes), kept at a temperature of 18°C., had been subjected to a preliminary treatment of darkness for 72 hours, they were introduced into a vessel cooled to 1°C., where they remained for a period of 4 hours. The eyes were then quickly excised in dim, red light, the operation not requiring more than 15 seconds, after which they were returned into darkness where fixation at approximately the freezing temperature ensued. The average measurements of the cone myoids in these preparations were as follows: 12 to 14 μ ; 10 to 15.4 μ ; 10 to 14 μ ; 10 to 11 μ ; 13 to 15.4 μ ; 9 μ . These values, although somewhat smaller than those given in table 10, can not be said to prove that low temperature shortens the myoids as does high temperature.

Gradenigro ('85) found that elevated temperature induced a shortening of the rod in the dark. After measuring the myoid length in a considerable number of preparations from retinas subjected to various temperatures both in light and in darkness,

I was unable to discover constant differences in length that could be correlated with definite temperatures. The agreement of mean values obtained from various retinas under identical temperature conditions was not good, and since the rod myoid measures only $6\ \mu$ to $12\ \mu$ in length, even small variations furnish serious obstacles in determinations of this kind. In any one preparation, moreover, variability in the length of adjacent myoids tended somewhat to mask a possible temperature effect. If anything, my measurements showed the reverse of what Gradenigro maintained, the elongation at 33°C. in the dark being greater than at 5°C. , but, as stated before, these results are by no means trustworthy.

A shortening of the rod through the action of high temperature, as claimed by Gradenigro, is of interest because, according to most investigators, light produces the same result. With this can be compared an analogous correlation in fishes, where light causes an elongation of the rod myoid and, as I have shown, elevated temperature does likewise. It is certain, on the contrary, that although the cones of both frogs and fishes shorten in the light, heating produces unlike responses in the dark.

(2) *Rana catesbiana* (larvae). Although the cones in both the 4.5 cm. and the 7.0 cm. larva of this frog are of large size, clearly defined temperature responses were not observed; indeed, the difference between the positions assumed even in light and darkness is not striking, the cone myoids in the light remaining well elongated in comparison to those of the adult *R. pipiens*. The variability in length in different preparations is considerable, yet if anything, the cones appeared more shortened at 3°C. than at higher temperatures. There is no marked shortening at 33°C. , for the cones under these conditions were as long as at lower temperatures, and in some cases longer. It is probable that the responses of the cones in adult *R. catesbiana* will be found to agree with those in other species which have been studied, although no experimentation was performed to determine this point.

3. *Necturus*. The rods, and the single and double cones of *Necturus* are very large, yet positional changes with varying tempera-

ture (3° to $28^{\circ}\text{C}.$) were not observed. Individual cells vary more or less in the height at which they are situated above the external limiting membrane, yet no constant differences of significant amount could be correlated with definite temperatures. With these results should be compared Garten's ('07) denial of a change in the position of the cones of the 'salamander' through the influence of light, such as Angelucci ('90) had previously claimed. Stort ('87), however, described movements in both the rods and the cones of Triton.

C. EXPERIMENTATION UPON EXCISED EYES

a. Effect of light and darkness

The results of a number of investigators since the first work of Englemann ('85) have indicated that the retinal elements of some vertebrates, and especially the frog, are subject to a nervous control, the action of which is not well understood.

The rôle which the nervous system plays either in producing or in assisting the movements of the various retinal elements is hard to demonstrate. Experimentation involving the direct action of light on excised eyes can not be expected to solve the problem decisively, for if no movements result, autoanaesthesia, or some similar disturbance due to the interrupted blood supply, may be the real cause. If, on the other hand, responses are called forth by direct stimulation, it by no means follows that a similar phenomenon necessarily occurs in the living animal, any more than a demonstration of the direct stimulation of muscle fibers proves that this rather than a nervous impulse is the normal method of muscle stimulation. The limitations which restrict a wide interpretation of results, however, do not lessen the interest involved in determining the extent to which the retinal elements can be directly stimulated.

Hamburger ('89) maintained that the cones and retinal pigment in excised eyes of the frog assumed the positions characteristic of light or darkness according to the conditions of the experiment. Dittler ('07) working on isolated frog's retinas obtained

a shortening of the cones in localized areas through the action of light but found darkness to be ineffectual. The spread of the response to portions of the retina unstimulated by light, led Dittler to investigate further the cause of cone retraction. He was able to furnish experimental proof that weak acids, resulting from catabolic processes in the retina, caused the cone myoid to shorten; hence he concluded that the cone myoid was not of itself 'lichtempfindlich,' as Englemann ('85) had believed, but was stimulated to movement through chemical agents resulting from the action of light on the retina. Fujita ('11), as a result of very limited experimentation, stated that the pigment of the excised eye of a frog expanded in the light but did not contract in the dark.

Ringer's solution, normal saline solution, and tap water were used by me for the immersion of excised eyes. When the first two media were employed the movements of the rods and cones of *Ameiurus*, through the action of light, were never clearly demonstrated; possibly such results are to be interpreted as evidence of a chemical control somewhat comparable to that described by Spaeth ('13) for the melanophores of *Fundulus*. Tap water did not inhibit the movements of any of the retinal elements of *Ameiurus* and consequently it was used in all subsequent experimentation.

The pigment of *Ameiurus*, *Abramis* and *Fundulus* did not contract when excised eyes from light-adapted fishes were subjected to darkness for periods of 4 hours or less. At most there was only evidence of a retraction of the distal accumulation of pigment, which is characteristic of light-adapted eyes, to form a more homogeneously pigmented zone (figs. 2, 10, 6). When the reverse experiment (subjection to light) was performed, the pigment of *Ameiurus* became maximally expanded in 2 hours (figs. 1, 3). Only the slightest tendency toward expansion, however, could be found after similar experimentation on the two other fishes. It thus appears that light acts directly on the pigment of *Ameiurus* only, while darkness is totally ineffective on all three animals.

When the rods and cones of *Ameiurus* and the cones only of *Abramis* and *Fundulus* were tested, the following results were obtained. The rods of *Ameiurus* moved both in light and in darkness, whereas the cones were stimulated only by light, no elongation occurring in the dark even when the experiment continued for 4 hours. The cones of *Abramis* and *Fundulus* did not change their positions to any extent either in light or in darkness. In the light the cones of *Abramis*, which were more carefully investigated than those of *Fundulus*, at most showed only slight retraction and never closely approached the external limiting membrane. If light did exert a direct influence on the cones or retinal pigment of this fish, the changes would be extremely easy to distinguish due to the wide difference between the light- and dark-adapted phases.

Since neither the cone cells nor retinal pigment of *Abramis* underwent movements under these conditions, it is possible that the accumulation of catabolic products, occasioned by the interruption of the blood supply, was responsible. Experimentation of the following kind shows the importance of maintaining the vascular circulation. If the optic nerve only of *Abramis* is cut, the retinal elements undergo their normal movements in darkness and in light. If, however, all the blood vessels and muscles are cut and the eye ball is attached to the body by the optic nerve only, no movements result. The objection may be made that some nervous mechanism is deranged by cutting these muscles and blood vessels, but this is hardly probable, as further experimentation, to be presented in a subsequent paper, on this and other fishes has shown.

In cases like the movements of the pigment or cones in excised eyes of *Ameiurus* through the action of light only, it is probable that an inhibitory tendency is also present, but the response to the stimulus furnished by light is sufficiently vigorous to overcome it. Either a less vigorous stimulus or response may explain why no movements of the cones and pigment occur in darkness.

An inhibition due to the presence of unremoved catabolic products, as postulated here, would be merely a form of auto-

anaesthesia. As will be shown, carbon dioxide and other anaesthetics do, in fact, arrest the movements of all the retinal elements of fishes.

Dittler ('07) accounted differently for the absences of elongation in the cones of isolated frog's retinas which were introduced from light into darkness. In order to appreciate his way of viewing this situation, it is necessary to understand the general theory advanced by him to explain the movements of the cones. In darkness an equilibrium was supposed to exist in the metabolism of the retina, the elongated cone myoid representing an unstimulated condition. Through the action of light, however, catabolic processes preponderate, and the accumulated acid wastes chemically stimulate the myoid to shorten. These conclusions were based upon experimental evidence by which it was shown that weak, free acids could be detected if isolated retinas were subjected to light in limited amounts of Ringer's solution, and further that such an acid solution was capable of causing other dark-adapted cones to shorten while still in the dark. To return to the case under consideration, Dittler believed that the accumulation of the catabolic products formed in the light merely continued its contractile influence after the isolated retina was removed into the dark, and since these products were not removed, the metabolic equilibrium could never be restored and consequently elongation failed to occur.

This theory of chemical stimulation is not supported by the condition in *Fundulus* and especially in *Abramis*, where the cones of excised eyes do not shorten even when exposed to light, for under these favorable conditions the tendency toward the production of a catabolic excess should be maximum. In still another way Dittler's theory does not explain a typical response of the cones of fishes. The cone myoid in isolated retinas of the frog shortens when the temperature is raised to 30°C. or more in the dark (fig. 36), and this fact Dittler used to support his view in the following logical manner. It is well known that most chemical reactions are accelerated by raising the temperature; hence in this case the autonomic equilibrium normally existing in the dark would become disturbed, the re-

sulting increase of catabolic products causing the cone myoid to shorten.¹⁴ Although Dittler's statement is not altogether clear, it seems evident that low temperature was supposed not only to reduce the metabolism of the retina to a low level, but also to render the cone myoid less 'empfindlich' to chemical stimulation.

The conditions in the dark-adapted cones of fishes, however, are entirely different, for here not only do the cone myoids elongate when the temperature is increased, but also elongated cones can be made to shorten by the use of low temperatures. If the shortening of the cones of fishes in the light were due to a chemical stimulation, how can the elongation of these elements in the dark through the action of heat be explained, since manifestly in this case the metabolic equilibrium tends to become destroyed, the result being the formation of an excess of catabolic wastes, which by analogy with the conditions in the frog, should cause the cone myoids to shorten? Moreover, the efficiency of low temperature in retracting elongated cones, and the correlation between the uniform degree of myoid elongation and the temperature gradient (p. 155) finds no explanation through Dittler's hypothesis.

It should be said, however, that Dittler strictly limited his conclusions, the experimental evidence for which appears to be well established, to the material upon which he worked, and was even reserved in suggesting the occurrence of a similar method of stimulation in living animals.

The reason why the rods of *Ameiurus* move in darkness, while the cones do not, may be as follows. The rod myoid normally shortens in the dark, whereas the cone myoid elongates. It is probable that the contractile function of the myoid is more vigorous than the reverse process of elongation. This is not only substantiated by the fact that dark adaption of the rod takes less time than light adaption, but also by experiments

¹⁴ Dittler did not formulate this conception in extenso as I have expressed it; yet several statements (pp. 317-318) show that this was his belief. A concluding quotation reads: . . . "der Einfluss der Temperatur überhaupt ganz nach physikalischen Modus zu Wirken scheint, und berechtigt uns, seine Wirkung rein in diesem Sinne zu fassen."

in which the optic nerve was cut. In such cases the rod never elongated in the light although it often showed a tendency to shorten when introduced into the dark, whereas the behavior of the cone cells in light and darkness was the exact opposite to that of the rod, since they tended to shorten in the light but remained unchanged in the dark. On these grounds, therefore, an explanation is offered to show why it is that through the strong stimulation produced by the direct action of light, both types of cells show characteristic responses, while in the dark only the more vigorous contractility of the rod myoid becomes effective. It must be remembered, however, that although the direction of the movements of the rod and cone cells are opposed, the real response of the protoplasmic myoid may be similar in both cases. If this were true, the apparent inconsistency in the movements of these elements would be due to a difference in the axis of contractility in the two kinds of myoids, and the explanation just advanced would not stand. Reference will be made to these possibilities in another place.

b. Effect of temperature

Previous attempts to determine the direct influence of temperature upon the retinal elements have been confined to the frog. Gradenigro ('85) found that if excised eyes of dark-adapted animals were subjected to a temperature of 30° to 36°C. the rods and cones shortened and the pigment expanded, both end-results being characteristic of light-adaption. Dittler ('07) was able to confirm Gradenigro's discovery concerning the cone cells. When the isolated retina was heated to 35° to 37°C. in the dark for 50 to 60 minutes, the cone myoids shortened. After retinas had been subjected to a temperature of 1° to 2°C. for many hours, on the contrary, no shortening of the cone myoid was observed.

The apparatus and methods used by me were similar to those described in connection with the experiments upon living fishes. The excised eyes were contained in test tubes which were suspended in jars of water kept at appropriate temperatures. Eyes

from the same animal were used simultaneously, one at each temperature extreme.

1. *Effect of temperature upon retinal pigment.* After many trials it was found that sharp differentiation of the retinal pigment of fishes was hard to secure at the extreme temperatures when the initial temperature had been intermediate. Accordingly, the expedient was employed of subjecting the living animals to a preliminary treatment either at 3°C. or at 25°C., and as a result uniformly satisfactory differentiation was obtained.

The retinal pigment of *Ameiurus* behaved precisely as in living animals. At a low temperature, both in darkness and in light (figs. 3, 1), the degree of distal migration was greater than that at a high temperature (figs. 4, 2). Particularly in experiments conducted in the light was this strikingly apparent, since in many preparations at 3°C. the pigment migrated so far distally that the more proximal portions of the cells were free of granules, while a sharp line of demarcation existed between the pigmented and non-pigmented zones.

A few experiments were made upon the dark-adapted eyes of *Abramis*. In this case also, a greater pigment expansion was found at 3°C. than at 25°C. (figs. 11, 12).

Reference has been made to the contention of many investigators that there is an apparent nervous control over the movements of the frog's retinal elements. It has been shown that at a medium temperature in the dark the pigment is maximally contracted (fig. 18), whereas at higher and lower temperatures (figs. 17, 19) a considerable degree of expansion is effected. Not only is the amount of migration occasioned by temperature much more extensive than that in fishes, but also the similarity between the effects of the two temperature extremes as contrasted with an intermediate temperature, has no parallel among other pigment cells or even in melanophores.

Since it is at least agreed that the pigment of excised eyes of the frog expands in the light, it ought to be possible to observe the effect of temperature, if this agent acts directly upon the pigment cells. Excised eyes from animals that previously had been at an intermediate temperature in the dark, were subjected

to temperatures of 3°, 16°, and 33°C., but no changes were observed in the position of the retinal pigment. These results, which do not agree with Gradenigro's statement, by no means furnish conclusive proof that in the living frog temperature operates through the nervous system, yet when supported by a comparative study of the retinal pigment and melanophores of other animals, the conclusion reached by Herzog ('05), that the expansion of the frog's retinal pigment under these circumstances is of nervous origin, involving the principle of specific energies, becomes highly probable.

According to Fujita ('11), when excised eyes from dark-adapted frogs are retained in the dark for 20 minutes, the pigment assumes a partial light position. My observations do not confirm this result, for no migration of any consequence occurred.

2. *Effect of temperature upon visual cells.* When a study of the visual cells of *Ameiurus* was made, the effect of temperature was found to be essentially similar to that upon normal animals.

When eyes from dark-adapted individuals of *Abramis* that had previously been kept at a temperature of 25°C. were likewise excised and subjected to 5°C. and 25°C. in the dark, those at 25°C. (fig. 27) retained the elongated position while those at 5°C. (fig. 25) shortened to a considerable extent although somewhat less than in living animals. Mention has already been made of the significance of these results in connection with the applicability of Dittler's theory of chemical stimulation to the cones of fishes. Although the rods of *Abramis* at both temperatures showed a distribution extending over wide limits, yet the shortest measured 12 μ at 5°C. as compared with 20 μ at 25°C., and the modal elongation at the same temperatures, as judged by the eye, was 18 μ and 25 μ respectively. It is interesting to note that in the excised eyes of dark-adapted *Abramis*, temperature is able to produce changes in both the retinal pigment and visual cells, notwithstanding the fact that light and darkness are wholly ineffectual in this respect.

Table 11 summarizes these results from typical retinas.

A few experiments were performed upon the cone cells of the frog. The results obtained were identical with those stated

TABLE 11

Measurements of the visual cells from the retinas of two Ameiurus and two Abramis, of which one of each had been kept at 5°C. and the other at 25°C. in the dark; the values are in micra and represent measurements taken along axes coinciding with radii of the eyeball

FISH	TEMPERATURE °C.	CONE MYOID	ROD INNER MEMBER
Ameiurus No. 1.....	5	4-16	5-6
Ameiurus No. 2.....	25	19-32	8-12
Abramis No. 1.....	5	10-30	18
Abramis No. 2.....	25	35-50	25

by Dittler ('07), who used isolated retinas. In dark-adapted eyes which were placed in water at a temperature of 33°C. the cone myoids shortened (fig. 36), while at 3°C. or 16°C. (figs. 34, 35) the myoids remained for the most part unchanged. These results are identical with those found by Fujita ('11) and myself on the cones of living animals, and indicate that, unlike the pigment cells, the movements of the cones are not dependent upon nervous control. If an influence of the nervous system over these elements exists in the normal animal, it is at least not manifested as is the control over the retinal pigment, in which changes at both high and low temperatures can be interpreted according to the principle of specific energies.

D. EFFECT OF ANAESTHETICS

Various instances have been noted throughout this paper in which the behavior of the retinal pigment and the visual cells, when deprived of their blood supply, cast suspicion upon auto-anaesthetization as being the factor causing suspension of movement. Certain conditions discovered in the responses of melanophores in the web of the frog's foot had previously suggested such a possibility; indeed, it was this difficulty which led to the abandonment of the frog's melanophore as material for an investigation somewhat similar to the present one. In this way my interest was aroused to determine the effect of anaesthetics on the movements of the retinal elements, both in normal animals and through the more direct action upon excised eyes.

The effect of certain drugs, as quinine and strychnine, upon the retinal pigment ('protoplasmagifte') is in dispute. It is clear, however, from the work of Ovio ('95) and of Lodato ('95) that cocaine can arrest pigment migration.

As a precaution against a possible source of error, animals were never introduced from one condition of light or darkness to the other without having been previously subjected to a brief preliminary treatment of the anaesthetic which was to be tested.

a. Retinal pigment

1. *Carbon dioxide.* The carbon dioxide used in these experiments was a commercial soda-water product sold under the trade name of 'Pureoxia.' Quantitative determinations of the concentrations used were made by titration with $\frac{N}{50}$ sodium carbonate, using phenolphthalein as an indicator.

In the first experiments made on *Ameiurus* the movement of the pigment was arrested by a strong solution of carbon dioxide, but since none of the animals survived such treatment the obvious objection exists that the pigment cells also may have been killed.

A slight refinement in method consisted of revivifying the fishes at intervals, by temporary removal to running water, until opercular movements were restored. By this method fishes were kept alive for 2 hours, during which time four or five revivifying treatments were necessary. The migration of retinal pigment was shown to be checked both in light and in darkness, yet controls proved that the cells were not permanently injured.

A method which gave more satisfactory results was devised after repeated trials had given a mixture of tap water and carbonated water of sufficient strength to anaesthetize an *Ameiurus* but not to prohibit opercular movements of greatly reduced amplitude. The record of an experiment will well illustrate both the method and the results.

Experiment 4.1.6. A dark-adapted *Ameiurus* was placed in a mixture of 1 part of 'Pureoxia' to 4 parts of tap water, and after re-

maining 10 minutes in the dark the jar was removed into strong diffuse daylight for $1\frac{1}{2}$ hours. During this time, the fish was practically motionless except for a very weak but rhythmical pulsation of the opercular rims. At the end of the experiment one eye was removed and fixed. The *Ameiurus* was allowed to recover until the next day when the other eye was removed. The pigment in the eye which had been subjected to carbon dioxide was in the typical dark position (cf. fig. 4) while the pigment of the control eye was maximally expanded (cf. fig. 2). Titration of the anaesthetizing solution showed that the concentration of carbon dioxide had been in the ratio of 60.14 cc. per litre of water.

In the converse experiment from light to darkness an *Ameiurus* lived 3 hours in a similar solution (53.07 cc. of carbon dioxide per litre) during which time the pigment retained its light distribution, whereas the control eye removed on the next day, showed maximal contraction.

These results prove conclusively that in the presence of certain concentrations of carbon dioxide the pigment cells are not injured but are in a condition of anaesthetization whereby there is a failure to respond to the normal stimulus causing contraction and expansion. Such experimentation, however, does not show whether this failure is due to a direct effect upon the pigment cells or to an inhibition through the central nervous system.

To demonstrate which alternative is true, the effect of carbon dioxide was tested on excised eyes of *Ameiurus*. If, under these conditions, a migration occurs a direct influence of the anaesthetic on the cell itself will be disproven, while on the other hand, if no migration ensues one can only infer that a similar direct action on the pigment cell is responsible for the whole course of events in the living fish, whereas an inhibition through the central nervous system may be involved as well.

For such an experiment the excised eye of *Ameiurus* is well adapted, since its pigment has been shown to migrate from the dark to the light position, although the reverse process does not occur. Excised eyes of dark-adapted fish were exposed to light in a solution of carbon dioxide having a strength of about 60 cc. per litre. The pigment in each case was arrested in the contracted position.

The work described for living *Ameiurus* has been repeated on both *Abramis* and *Fundulus* with identical results. In every case the pigment maintained the position it occupied previous to the application of the anaesthetic.

The results obtained in this study, as a whole, are very different from those of Fick ('90), who concluded that the retinal pigment of the frog expanded when the animals were subjected to an atmosphere of carbon dioxide gas. Fick attributed this result to asphyxiation and it is certain that the experimental conditions in his work differed greatly from those in my tests. In order to make the experiments more comparable, frogs should be treated with a mixture of oxygen and carbon dioxide gases in which they could live.

2. *Ether*. The anaesthetic effect of ether on the retinal pigment was demonstrated by a series of tests that duplicate those described with carbon dioxide. Care must be observed against using an excess of ether since otherwise a partial or complete disintegration of the pigment cells results.

Both dark and light trials were made on *Ameiurus*, *Abramis*, and *Fundulus*. In each animal the pigment was found to be completely arrested in whatever position it occupied at the beginning of the experiment. Controls proved that ether, if used in small amounts, does not permanently injure the pigment cells.

Ether also checked the migration of pigment in excised eyes of dark-adapted *Ameiurus* when such eyes were subjected to light.

3. *Chloretone and urethane*. These substances are such satisfactory narcotizing agents that their effect was tested upon the retinal pigment of *Ameiurus*. Individuals lived in 0.1 per cent chloretone or in 1.0 per cent urethane, but the pigment was not arrested in its movement from the dark to the light phase. In concentrations of 0.5 per cent chloretone and 2.5 per cent urethane, the pigment migrated when fish were brought from darkness to light although the animals died in both cases.

The results from all the foregoing experimentation are of interest in showing the difference in the effect upon pigment cells of four powerful anaesthetics, of which only two were

efficient. The experiments with chloretone and urethane also prove that even though the animal as an organism dies, the pigment, nevertheless, can expand independently.

b. Visual cells

Experiments similar to those just described were repeated in order to determine the action of anaesthetics on both rod and cone cells. Since the cone myoid is maximally elongated at about 25°C. in the dark (figs. 25, 27), this condition was taken advantage of in producing sharp contrasts between dark and light phases. The cones of *Abramis* and *Fundulus*, on account of their great contractility, were particularly favorable for observation, as were the rods of *Ameiurus* because of their large size.

The results of these experiments are shown in table 12.

TABLE 12

A tabulation of the effects of carbon dioxide and ether upon the movements of the visual cells of Ameiurus, Abramis, and Fundulus; X indicates that the movements of the elements were completely arrested; conditions corresponding to the blank spaces were not investigated

FISH	CONE		ROD	
	Dark to Light	Light to Dark	Dark to Light	Light to Dark
<i>Ameiurus</i>			X	X
<i>Abramis</i>	X	X	X	X
<i>Fundulus</i>	X	X		

The conclusion is, therefore, that both ether and carbon dioxide anaesthetize the visual cells of normal fishes to such an extent that neither light nor temperature is effective in causing positional changes.

A few experiments upon the excised eyes of *Ameiurus* showed that both carbon dioxide and ether have the same anaesthetic effect on the rods and cones as that described for normal animals.

Whether or not autoanaesthetization prevented movements of the retinal elements, as was suspected in previously described experiments when the normal blood supply was interrupted, it is at least demonstrable that certain anaesthetics do act in a

similar way. The effect of carbon dioxide is especially interesting for, as it is the commonest catabolic product, it may have been the agent that prevented movements in those cases. This conception is opposed to Dittler's ('07) view, which assumes the existence of a balance in the metabolism of the unstimulated cone cells which is disturbed by the increased catabolism through the action of light. The movement of the cone cells in the isolated retina of the frog was stated by Dittler to be due to the action of a weak free acid, the product of increased catabolism. This conclusion, which was supported by experimental evidence, is opposed to that postulated by me; nevertheless, it must be pointed out that the responses of the retinal elements differ considerably in fishes and in the frog, and while evidence for autoanaesthetization is indirect yet the results obtained from experimentation upon fishes can be consistently interpreted in this way, whereas Dittler's hypothesis does not meet all the known facts. A discussion of these points was given in another section of this paper.

E. EFFECT OF OXYGEN

Spaeth ('13) showed that the isolated melanophores of *Fundulus* contract in the absence of oxygen, but contracted melanophores do not expand when oxygen is the only stimulating agent present. Fick ('90) deprived dark-adapted frogs of oxygen by submergence in water or by introducing them into an atmosphere of hydrogen or carbon dioxide. As a result of this treatment he asserts that the retinal pigment underwent expansion. Dittler ('07) states that in frogs which are about to hibernate the cones are never as fully elongated as in active animals; but after subjection to an atmosphere of pure oxygen the cones can again be obtained in the maximal dark position. It seems probable that in this case the effect of oxygen was indirect, and the increased activity of the cone cells accompanied *pari passu* the return of other body activities.

In order to test whether or not the amount of oxygen available to a fish in any way controls the distribution of its retinal

pigment, a series of experiments, chiefly upon *Ameiurus*, were performed.

In experiments involving a reduced oxygen supply, the apparatus was simple. A $3\frac{1}{2}$ litre flask was filled with boiling water. The flask was then closed with a three-hole rubber stopper through which passed, (1) a glass tube extending to the bottom of the flask, which served to introduce gas from a hydrogen generator, (2) a glass overflow tube extending about three-quarters of the way down the flask, which served chiefly as an outlet for the hydrogen gas, (3) a mercury pressure regulator. As soon as the flask of boiling water was stoppered, the hydrogen supply was turned on and as a result, water was forced to escape through the overflow tube, its place being taken by hydrogen gas. When the water level reached the bottom of the overflow tube no more escaped, but the gas after bubbling through the water did so and was conducted through a water trap to the outside air. As the water cooled down to room temperature it could not take up oxygen since none was present, and furthermore, the bubbling hydrogen gas tended to expel mechanically any residual oxygen present in the boiled water.

Water containing an excess of oxygen was prepared by bubbling oxygen gas through water in a flask similar to that described in the former experiment, whence it escaped by means of an overflow tube leading into a water trap. The water used had previously been boiled and reoxygenated by an aquarium aerating device.

Quantitative determinations of the oxygen content were made at the expiration of all experiments by the method of Winkler (Treadwell and Hall, '05).

Ameiurus was used in most of the experimentation, although *Abramis* served in a few cases. The description which follows applies particularly to *Ameiurus*.

It was possible to reduce the oxygen supply to an amount in which the fish could not live.¹⁵ This, for example, happened

¹⁵ The normal oxygen saturation of water at 20°C. is 6.356 cc. per liter (Treadwell and Hall, '05). Boiled water which had been cooled rapidly was found to contain about 0.93 cc. per liter.

when only 0.8 cc. of oxygen per litre was present. On the other hand, in water containing an excess of oxygen (7.5 cc. per litre) respiratory movements of the operculum ceased, the fins appeared reddish in color and respiration may have been largely cutaneous.

In parallel experiments conducted both in the dark and at various light intensities, no difference could be detected in the positions of the pigment or visual cells under the extreme conditions of oxygen supply. This is not surprising, for presumably but little oxygen is needed to permit the cells to function, and since for the success of the experiment, the animal must have enough oxygen with which to keep itself alive, a crucial test involving a complete elimination of oxygen is not possible. Since the pigment can not be made to contract in excised eyes, but only to expand, a decisive experiment in which all oxygen might in this way be eliminated (similar to Spaeth's work on isolated chromatophores) was impossible.

Pigment, rods, and cones respond in a normal fashion when brought from darkness to light or vice versa in water containing the minimum oxygen content, about 0.9–1.0 cc. per litre, in which the *Ameiurus* can live.

Since no indication was observed of a tendency toward expansion in the retinal pigment cells of fishes which were deprived of oxygen, it is evident that the expansion described by Fick ('90) in dark-adapted frogs whose respiration rate had been reduced by covering the head with a velvet hood, is exceptional. In view of the well known respiratory function of the frog's skin it is possible that Fick's results are open to other interpretations, especially since his experiment, a repetition of the earlier work of Englemann ('85), is not in agreement with the latter's conclusion relative to the absence of movement in the retinal elements when frogs provided with velvet hoods were retained in the dark as controls to other experiments.

The chief value, therefore, of the work done by me is to show that within normal experimental limits the retinal pigment and visual cells of fishes are not affected by an increased or diminished oxygen supply.

F. INTERRELATION OF INTEGUMENTARY PHOTO-RECEPTORS AND
RETINAL ELEMENTS

The skin of several lower vertebrates has been shown to be sensitive to light. Among the fishes, Eigenmann ('00) stated that certain blind forms living in caves gave motor responses when stimulated by light, the photo-receptors presumably being located in the skin. Parker ('05) followed up some negative results obtained by one of his students on *Fundulus* by an investigation on ammocoetes, and proved that the integumentary nerves were sensitive to light, causing movements of the animal that were both 'phototropic and photodynamic.' A photo-receptivity of the skin of certain other vertebrates was first demonstrated by the following workers: Graber ('84) on Triton; Dubois ('90) on *Proteus*; Korányi ('92) on the frog; Carleton ('03) on *Anolis*; and Eycleshymer ('08) on *Necturus*.

Englemann ('85) covered the heads of dark-adapted frogs with a velvet cap and exposed the bodies to sunlight. Under these conditions, he asserted that in 15 minutes the pigment and cone cells assumed the maximal light position, whereas the same elements in control experiments conducted in the dark remained unchanged. Illumination of the skin for longer periods was said to result in a falling off ('herabsteigen') in the expansion of the retinal pigment and to a weakened response on the part of the cones. From these results he concluded (p. 507): "... dass Zapfen und Pigment des Auges von entfernten Körpergegenden aus reflectorisch in Bewegung gebracht werden können."

Fick ('90), in repeating this experiment of Englemann, found the pigment in an expanded condition while the frogs, ready for the test, were still in the dark, and after supplementary experimentation of various kinds he decided that pigment expansion accompanied disturbed respiration. In the case under consideration the velvet hood was supposed to have caused partial asphyxiation.

Korányi ('92) refers to the similarity in the responses of the retinal pigment resulting either from the illumination of the retina or of the skin only, yet he does not state that he actually observed this condition himself.

More recently Fujita ('11) asserted that after the head and forward extremities were bound with wet black cloth and the rest of the body was exposed to sunlight for 15 to 20 minutes, the 'eyes' remained in the dark-adapted condition.

I, myself, before learning of Fujita's work, had performed several experiments of the same kind. The head and fore body of dark-adapted frogs were bandaged with many thicknesses of black velvet and the remainder of the body and the hind legs were exposed to direct or diffuse sunlight for periods of 15 minutes to 1 hour. Care was taken to keep the skin moist and to guard against the heating tendency of direct sunlight by using a heat filter. Although the animals were in an active condition at the end of the experiment, there was in no case a distinct change in the position of the cone cells or retinal pigment as Englemann maintained.

In an unpublished investigation by Mr. S. G. Wright, no direct responses were observed when *Ameiurus*, from which the eyes had been removed, were illuminated with the light of an electric arc. The normal fish, as is well known, is a night feeder, yet it also frequented equally the light and dark halves of an aquarium jar.

It is, conceivable, however, that the soft skin of this animal contains a photoreceptive mechanism, even though the motor responses to light fail to indicate its presence. One of the possible ways in which an integumentary photosensitivity could be manifested is through an influence on the position of the retinal elements, similar to the relation which Englemann believed to exist in the frog. In view of such a possibility several experiments were performed in which dark-adapted fish with bandaged heads were exposed to daylight and to the light of an electric arc for periods of 45 minutes to 1 hour. In no case, however, was there the slightest tendency toward movement on the part of the rods, cones, or retinal pigment.

These results indicate that neither in the frog nor in *Ameiurus* are movements of the retinal pigment or visual cells evoked by a coöperation of dermal photosensitivity and 'retino-motor' nerve fibers. Consequently, Englemann's experiment does not

have the significance that he believed it possessed, when he attempted to furnish physiological proof that in the frog: "Jedenfalls aber laufen . . . auch *retinomotorische Fasern* von den grossen Nervencentren aus durch den Sehnerv zum Auge" ('85, p. 506).

4. DISCUSSION

We have so accustomed ourselves to view the phenomena exhibited by living organisms from the evolutionary standpoint that an 'explanation' which will reveal the adaptiveness of an organism to its environment is demanded whenever a system of relations involving constant responses to definite stimulating agents is discovered.

To make dogmatic assertions regarding the presence or absence of adaptation in a set of responses is, obviously, a matter of exceeding danger, yet if the phenomena exhibited by a series of representative animals to definite stimulating agents are shown to be variable, it is at least evident that a single inclusive explanation will not be forthcoming.

The writer has attempted to show elsewhere (Arey '15) that the discontinuous occurrence of photomechanical responses in the visual cells and retinal pigment, both in the various vertebrate classes and among different representatives of certain individual classes, renders it extremely difficult from the adaptational standpoint to devise a satisfactory explanation for the meaning of these movements. The majority of theories which attempt to link the known responses of the retinal elements with the mechanism of light perception are, without doubt, highly speculative, and since for the most part they lack an experimental basis of any kind, they must remain of interest only as ingenious and interesting possibilities. In the case of the retinal pigment at least, it is possible to compare the responses to light and darkness with those exhibited by melanophores in general (Parker '06, p. 413), and from our present knowledge we are unable to see either in the reactions of the retinal pigment or in those of the rods and cones anything more

than the presence of constant protoplasmic responses to a definite stimulating agent.

When the effect of temperature is considered, a lack of uniformity at once becomes apparent.

Among the fishes the effect of temperature upon the retinal pigment is in agreement with Parker's ('06) general conclusion that in all melanophores, low temperature has the same effect as light and high temperature the same effect as darkness.¹⁶ In the retina of the living frog where this statement holds true only between the temperatures of 0° and 19°C. in the dark, notwithstanding the identical photic responses of the retinal pigment in fishes and amphibians, it is probable that a nervous control is responsible for the expanded condition both at low and at high temperatures; hence the responses in this animal are not comparable to those in fishes. Since only a limited temperature reaction occurs among fishes, and this in darkness as well as in light, it probably has no immediate adaptive significance, but merely represents the survival of a tendency shown in the responses of melanophores in general.¹⁷ In homothermous animals temperature, of course, can play no part in the normal activity of the retinal pigment, and even if the temperature responses in poikilothermous animals have an adaptive significance, it is evident that this particular advantage must be unavailable to warm blooded vertebrates, in which keenness of sight is best developed.

Moreover, the action of temperature upon the cone cells in the dark is variable. In the fishes, the cone myoids greatly elongate when warmed and shorten when cooled, while in the frog the cones are maximally shortened at high temperatures only and at all other temperatures remain unchanged.

The inconsistent action of temperature in producing movements of the visual cells apparently has no common adaptive

¹⁶ It should be pointed out, however, that in the case of the melanophores of the frog's skin, the reverse of this statement is true, since high temperature produces similar effects to light, and low temperature to darkness.

¹⁷ Below 38°C. temperature has practically no effect on the rate of decomposition of the visual purple, as Kühne ('79) showed, hence the changes of the pigment through the action of temperature are not related to this phenomenon.

value, and, moreover, as in the case of retinal pigment, variable temperature can play no part in the normal movements of these cells in homothermous animals.

It may be asked how it happens that high temperature in the dark produces diametrically opposed results upon the cone myoids of fishes and of the frog, when the responses to light are identical. The nervous system, presumably, is not involved in these reactions since temperature has a similar effect upon excised eyes. Between the minimal and optimal limits the movement of undifferentiated protoplasm is uniformly accelerated with increased temperature, and as has been shown in another place (p. 135) the responses in length of the cones of *Abramis* are markedly similar in this respect. Whatever the details of the process may be, it seems evident that among the fishes temperature acts purely as a physical agent in controlling the velocity of the reactions leading to positional changes of the cones. In frogs temperature apparently acts in an entirely different manner. The fact that only high temperature is effective in producing a change, a shortening of the myoid, is best explained on the basis of Dittler's theory of chemical stimulation, whereby increased temperature can be conceived of as favoring the formation of catabolic wastes which chemically stimulate the cones to shorten, while low temperature probably acts both in retarding catabolism, and by reducing the sensitivity of the myoid toward such products as are formed even under these conditions.

A comparative study of the responses of the visual cells, throughout the various classes, to light and temperature reveals difficulties in explaining the mechanism by which their positional changes are accomplished. Why do the rod cells of some animals shorten in the light whereas others lengthen? Why do the rods of fishes and of birds lengthen through the action of light, whereas the cones shorten?

From the physiology of simple protoplasm, two alternatives are open. Either the myoids of the visual cells have become specialized to respond to certain stimulating agents in different ways, or, in various retinas the morphological structure of these

contractile regions is such, that although the protoplasm responds similarly in all cases, the visible result upon the position of the entire cell is variable. The latter alternative would be realized if the active protoplasm were differentiated into analogues of myofibrillae which were arranged in some cases axially in the myoid and in other cases transversely or spirally. To become effective in elongating the myoid through contraction, a simple spiral 'myofibril' would have to make an angle greater than 45 degrees with the long axis of the myoid.¹⁸

In attempting to interpret the adaptiveness of the movements of the vertebrate retinal elements, it is evident from the foregoing discussion that neither with respect to the action of temperature nor of light has a satisfactory and constructive conclusion been reached. From the present state of our knowledge, therefore, the situation may be summarized in the following way. Although the movements of the visual cells and retinal pigment, when present, may have a certain unknown significance in connection with the mechanism of light perception, such movements can be interpreted at present only in terms of protoplasmic responses to definite stimulating agents.

5. SUMMARY

1. The retinal pigment of the fishes studied requires 45 minutes to 1 hour for light adaption and from 30 minutes to 1 hour for dark adaption. The cones of *Abramis* assume the light (shortened) position in 45 minutes, the dark (elongated) position in 30 minutes. Maximal elongation of the rods of *Ameiurus* in the light occurs in 45 minutes, maximal shortening in darkness in 30 minutes.

2. Both in light and in darkness, the retinal pigment of fishes shows greater expansion at a low than at a high temperature. High temperature is apparently more efficient in causing this

¹⁸ In connection with these possibilities mention will be made of only the fibrils found by Hesse ('04) in both the inner and outer members of the rods and cones of several vertebrates, and of the longitudinal fibrils which Howard ('08) was able to trace throughout both the rods and cones of *Necturus*. Both workers, however, considered such structures as neuroid in character.

redistribution of pigment than is low temperature. The results obtained from the four fishes studied indicate that extreme conditions both of expansion and of contraction are not to be found in the retinal pigment of any one fish.

3. In darkness, the retinal pigment of the frog undergoes striking expansion between the temperatures of 0° to 14°C. and 19° to 33°C., whereas at the intermediate temperatures of 14° to 19°C. it is highly contracted. Temperature is without effect upon light-adapted retinas. Since the retinal pigment of the larvae of *Rana catesbiana* shows temperature responses identical with those characteristic of adults, there is no temporary larval recapitulation of the responses characteristic of fishes.

4. Light and darkness produce but limited changes in the distribution of the retinal pigment of *Necturus*. No definite effect of temperature could be detected. It is probable that the peculiar responses found in the frog have been developed within the anuran group.

5. The cone myoids of fishes shorten at low temperatures in the dark; at high temperatures they lengthen. Not only is elongation progressive, extreme conditions being found at 0° and 30° ± C., but also the rate of change is directly proportional to the temperature gradient. Temperature is ineffectual in the light.

6. The myoid of the rods of fishes also elongates at high temperatures and shortens at low temperatures, but the extent of change is much less than that of cone cells.

7. The cone myoid of adult frogs in the dark shortens when subjected to a temperature of 19° to 33°C., but remains elongated at all lower temperatures. No definite temperature responses of the cone myoids were found in the larvae of *Rana catesbiana*.

8. A correlation between the length of the rod myoid and temperature was not detected in the frog either in darkness or in light.

9. The positions of the visual cells of *Necturus* are not affected by temperatures between the limits of 0° and 28°C.

10. In excised eyes of the four fishes studied, light causes a migration of the retinal pigment in *Ameiurus* only, whereas the pigment of none of the fishes moves in darkness. The rods of excised eyes of *Ameiurus* undergo movements both in the light and in the dark, the cones move in the light only. Neither exposure to darkness nor to light produces positional changes in the cone cells of excised eyes of *Abramis* or *Fundulus*. Where tested, temperature was found to cause movements in the retinal pigment and cone cells in the excised eyes of fishes.

11. It is probable that the absence of responses in the excised eyes of fishes is due to an autoanaesthetization caused by the accumulation of catabolic products.

12. Dittler's theory of the chemical stimulation of the cone myoid, propounded to explain the movements of the cone cells in isolated frog's retinas, does not satisfactorily meet many conditions found in the responses of the cones of fishes.

13. The effects of temperature upon the rods, cones, and retinal pigment of the excised eyes of fishes are identical with those found in living animals, hence it is probable that temperature has a direct action upon these elements, its effect being physical in the sense that the chemical activity of the protoplasm is thereby accelerated to varying degrees.

14. Temperature has no effect upon the retinal pigment of the excised eye of the frog, therefore it is plausible that the action of temperature in living animals is physiological, whereby any adequate stimulus acting through the central nervous system can produce a striking pigment expansion according to the principle of specific energies. As in living animals the cone cell of the excised frog's eye responds by a shortening at an elevated temperature only; it is probable that temperature acts directly upon the cone myoid, for this response, unlike that of the pigment, can not be interpreted by the principle of specific energies.

15. Neither in the frog nor in *Ameiurus* are movements of the retinal elements evoked by exposure of the skin only to light. Hence the existence of an interrelation between dermal photosensitivity and the responses of the retinal elements by

means of 'retino-motor' nerve fibers, as maintained by Englemann, is not substantiated.

16. Within the experimental limits at which fishes can be kept alive, the retinal pigment and visual cells are not affected by an increased or diminished oxygen supply.

17. Both in darkness and in light, and in excised as well as in normal eyes, carbon dioxide and ether completely check the movements of all the retinal elements of fishes. Chloretone and urethane, on the contrary, are inefficient in this respect. The action of carbon dioxide suggests that this may be the catabolic product that in many cases restrains the movements of the retinal elements when the circulation of the blood is interrupted.

18. Although the movements of the visual cells and retinal pigment, when present, may have a certain unknown significance in connection with the mechanism of light perception, such movements can be interpreted at present only in terms of protoplasmic responses to definite stimulating agents.

Cambridge, Mass., April 10, 1915.

POSTSCRIPT

A study of the influence of light on the movements of the frog's rod has just been completed by the writer. Careful measurements prove that these elements are extended in the light and are retracted in darkness. Hence the results of the older workers (p. 123), who believed that the photic responses of the frog's rod-myoid are the reverse of those occurring in fishes and birds, are not substantiated.

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1887 Mouvements des éléments de la rétine sous l'influence de la lumière. *Arch. néerlandaises des Sciences exact et naturelles*, publ. p. 1. *Soc. holland. des Sciences*, Tom. 21, Livr. 4, pp. 316-386.
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EXPLANATION OF PLATES

The figures of Plates 1 to 4 are photomicrographs; the figures of Plate 5 were drawn with the aid of a camera lucida.

ABBREVIATIONS

<i>bac.</i> , rod	<i>my.con.</i> , cone myoid
<i>con.</i> , cone	<i>pd.cl.pig.</i> , base of pigment cell
<i>con.acc.</i> , accessory cone	<i>prs.dst.bac.</i> , rod outer member
<i>ell.bac.</i> , rod ellipsoid	<i>prs.dst.con.</i> , cone outer member
<i>ell.con.</i> , cone ellipsoid	<i>rtn.</i> , retina
<i>gtl.ol.</i> , oil globule	<i>scl.</i> , sclera
<i>mb.lim.ex.</i> , external limiting membrane	<i>st.nl.ex.</i> , external nuclear layer
<i>my.bac.</i> , rod myoid	<i>st.pig.</i> , pigment layer

PLATE 1

EXPLANATION OF FIGURES

The photographs in this plate, which show the influence of temperature on the distribution of the retinal pigment of fishes, are all at a magnification of 170 diameters.

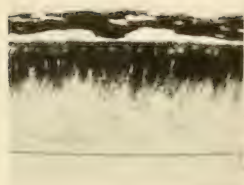
1. *Ameiurus*, 5°C. in the light.
2. *Ameiurus*, 25°C. in the light.
3. *Ameiurus*, 5°C. in the dark.
4. *Ameiurus*, 25°C. in the dark.
5. *Fundulus*, 5°C. in the light.
6. *Fundulus*, 25°C. in the light.
7. *Fundulus*, 5°C. in the dark.
8. *Fundulus*, 25°C. in the dark.



1



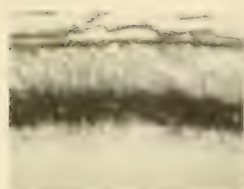
2



3



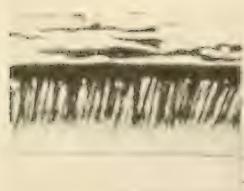
4



5



6



7



8

PLATE 2

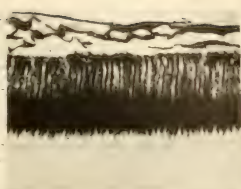
EXPLANATION OF FIGURES

The photographs in this plate, which show the influence of temperature on the distribution of the retinal pigment of fishes, are all at a magnification of 170 diameters.

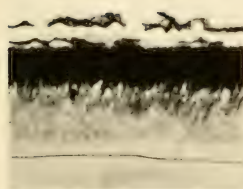
- 9 Abramis, 5°C. in the light.
- 10 Abramis, 25°C. in the light.
- 11 Abramis, 5°C. in the dark.
- 12 Abramis, 25°C. in the dark.
- 13 Carassius, 5°C. in the light.
- 14 Carassius, 25°C. in the light.
- 15 Carassius, 5°C. in the dark.
- 16 Carassius, 25°C. in the dark.



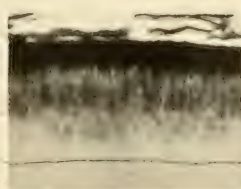
9



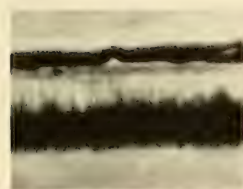
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16

PLATE 3

EXPLANATION OF FIGURES

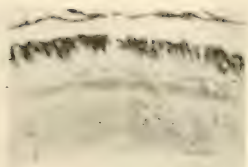
Figures 17 to 22, which are photographs showing the influence of temperature on the distribution of the retinal pigment of the frog (adults and larvae), are all at a magnification of 170 diameters. The larval *Rana catesbiana*, from which figures 20 to 22 were made, had a total body length of 7.0 cm. A Leitz $\frac{1}{2}$ homogeneous immersion objective was used in making figures 23 and 24, which are magnified 715 diameters.

- 17 *R. pipiens* (adult), 3°C. in the dark.
- 18 *R. pipiens* (adult), 18°C. in the dark.
- 19 *R. pipiens* (adult), 33°C. in the dark.
- 20 *R. catesbiana* (larva), 3°C. in the dark.
- 21 *R. catesbiana* (larva), 16°C. in the dark.
- 22 *R. catesbiana* (larva), 32°C. in the dark.
- 23 *Carassius*, 27°C. in the dark.
- 24 *Carassius*, 3°C. in the dark.

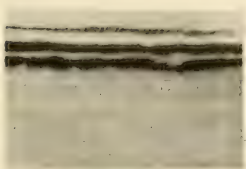


17

sel.
st. pig.
st. bac. con.
ret.



20

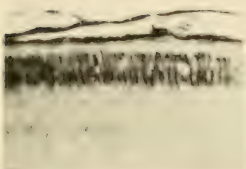


18

sel.
st. pig.
st. bac. con.
ret.

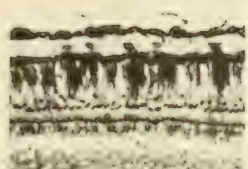


21



19

sel.
st. pig.
st. bac. con.
ret.

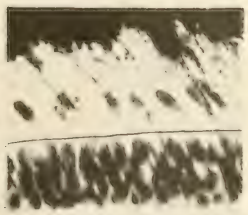


22



23

con.
st. pig.
bac.
mb. lim. ex.
st. nl. x.



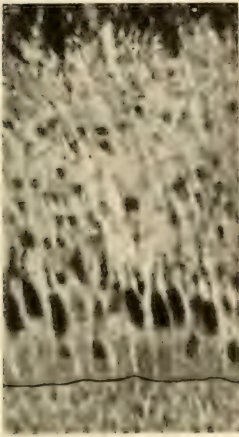
24

PLATE 4

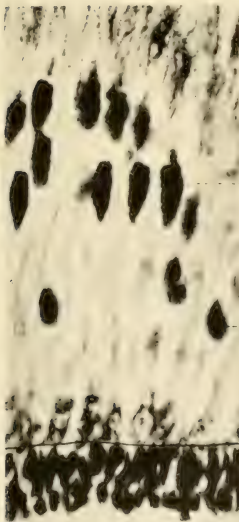
EXPLANATION OF FIGURES

These photographs, taken with a Leitz $\frac{1}{12}$ homogeneous immersion objective, are at a magnification of 715 diameters and show the responses of the myoids of the cone cells of fishes when under the influence of temperature.

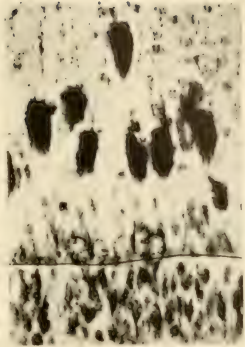
- 25 Abramis, 3°C. in the dark.
- 26 Abramis, 15°C. in the dark.
- 27 Abramis, 27°C. in the dark.
- 28 Fundulus, 3°C. in the dark.
- 29 Fundulus, 27°C. in the dark.



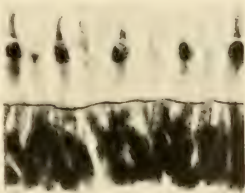
25



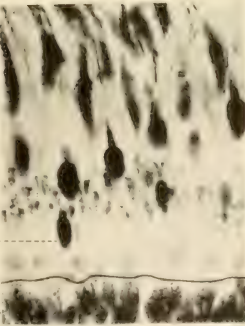
27



26



28



29

PLATE 5

EXPLANATION OF FIGURES

All drawings in this plate are at a magnification of 930 diameters, a Leitz $\frac{1}{2}$ homogeneous immersion objective being used.

30 Showing the positions of the rods and cones in a typical light-adapted retina of *Ameiurus*.

31 Showing the positions of the rods and cones in a typical dark-adapted retina of *Ameiurus*.

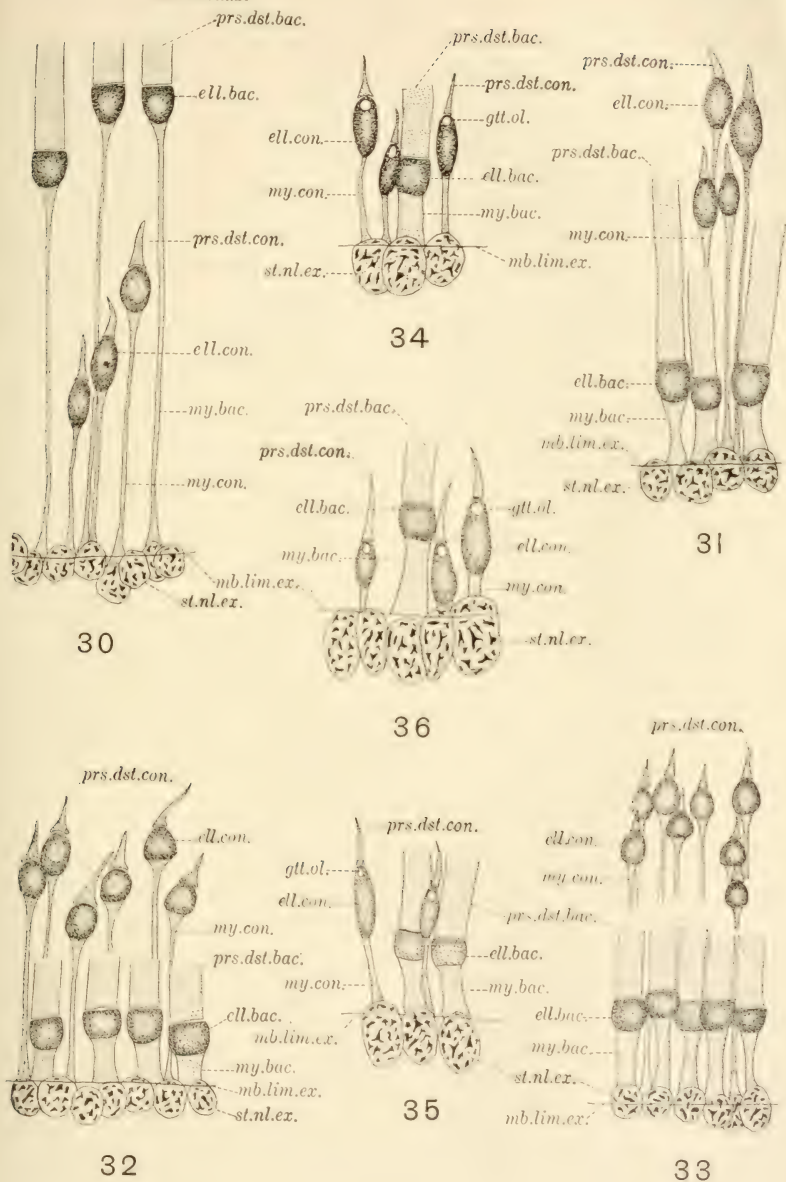
32 Showing the effect of low temperature (3°C.) on the position of the dark-adapted rod- and cone-cells of *Ameiurus*.

33 Showing the effect of high temperature (27°C.) on the position of the dark-adapted rod- and cone-cells of *Ameiurus*.

34 From the retina of a *Rana pipiens* that had been previously kept at a temperature of 3°C. in the dark. The cone myoids remain elongated as at an intermediate temperature.

35 From the retina of a *Rana pipiens* that had previously been kept at 18°C. in the dark. The cone myoids are elongated.

36 From the retina of a *Rana pipiens* that had been kept at 33°C. in the dark, showing the resulting shortening of the cone myoids.



REGENERATION IN THE BRAIN OF AMBLYSTOMA

I. THE REGENERATION OF THE FOREBRAIN

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FOUR FIGURES

Up to the present time the data dealing with regeneration in the central nervous system have been exceedingly conflicting. The early workers reported the regeneration of various definitive parts after they were extirpated. As far back as 1890 Danielewsky found that the removal of the cerebral hemispheres of the frog resulted in the formation of a 'cerebral mass' which he believed contained embryonic nerve cells, though this mass was in no way a new hemisphere. More recently Bell, in 1907, removed the 'lateral half' of the brain of the frog and found that the brain nearly always is reformed though never does it reach the normal size.

On the other hand, Schaper ('98) found that the removal of portions or of the entire brain of *Rana esculenta* was never followed by regeneration. Rubin in 1903 working with *Rana fusca* larvae found that no regeneration occurred after the removal of the brain or of parts of it.

The results of experiments published elsewhere (Burr '16) show conclusively that the nasal placode of *Amblystoma* and also of the frog does not regenerate when it is completely removed. In addition, the large amount of work by Lewis and others has shown that the eye will not regenerate when all of the anlage is removed. It was deemed probable, therefore, that complete extirpation of the cerebral hemisphere would not be followed by even a partial regeneration of the part removed. The discrepancy in the results previously reported might be due to incomplete operations. The regeneration of parts of

the brain would then be due to the fact that the entire substance of the hemisphere was not removed, enough being left to carry the regeneration through to a considerable degree of completeness. A condition similar to this has been shown by Harrison to exist in the extirpation of limb buds in *Amblystoma* (Harrison '15).

So far as it has been possible to ascertain, no attempt has previously been made to control the stimulus afforded by the functional activity of the end organ normally connected with the part of the central nervous system removed. Extirpation of a portion of the brain has invariably carried with it the removal of the end organ. It is a little difficult then to see why a part should regenerate when its activity has ceased owing to the removal of the end organ.

In order to obtain some answer to the above question the following experiments were performed on *Amblystoma* larvae. Two series of operations were undertaken on embryos possessing neither peripheral nerves nor a circulatory system. In the first of these the right cerebral hemisphere and the right nasal placode were extirpated, the cut that severed the hemisphere from its connections passing directly in front of the optic stalk. In the second series the right telencephalon was removed but the right nasal placode was left in position. This was accomplished by turning back the flap of skin containing the placode and removing the underlying forebrain. The flap was then returned to its former position and held in place until the wound had healed. Great care was taken to remove all of the cells of the cerebral hemisphere in all the operations.

The above experiments subject the brain tissue left by the extirpation of the hemisphere to two conditions. In the first series of operations the nervous tissue is left to regenerate without the possibility of any stimulus from the end organ that is normally connected with it. In the second series the end organ, the nasal placode, is left in its normal position and may therefore act as a stimulus to the nervous tissue (Burr '16).

In the first series of experiments in which the right hemisphere and the right nasal placode were removed the wound usually

healed within the first twenty-four hours. Five days after the operation a new wall had formed connecting the wall of the right diencephalon with the wall of the left telencephalon. At first this wall consists of a narrow band of new cells bridging the interventricular foramen. As growth proceeds the narrow

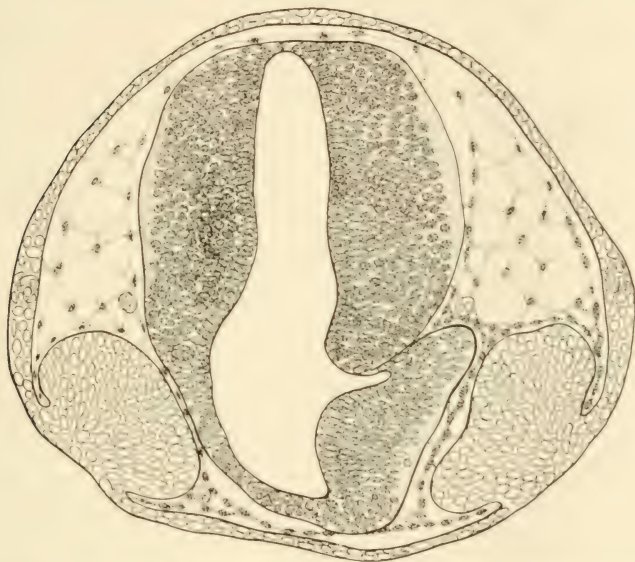


Fig. 1 Transverse section of embryo five days after operation, by which the cerebral hemisphere was removed, leaving the nasal placode in place, showing curtain of cells across the interventricular foramen. $\times 50$.

band of cells is drawn out into a thin plate, never more than two or three cells thick, stretching across the foramen (figs. 1 and 3).

A careful inspection of a series of operated larvae makes it quite clear that the new tissue thus formed is derived from the primary ependymal cells that line the neural tube. Figure 2 drawn from a section of an operated larva of the second series shows the origin of this new tissue from the margin of the dien-

cephalon. It is composed entirely of the typical columnar cells that make up the ependyma. As growth proceeds the cells of the new membrane lose their columnar shape and become metamorphosed into flattened quadrilateral cells. In the three months old larva, a section of whose brain is shown in figure 3, it is evident that this metamorphosis is accompanied by a thinning of the tissue so that at this time the membrane is

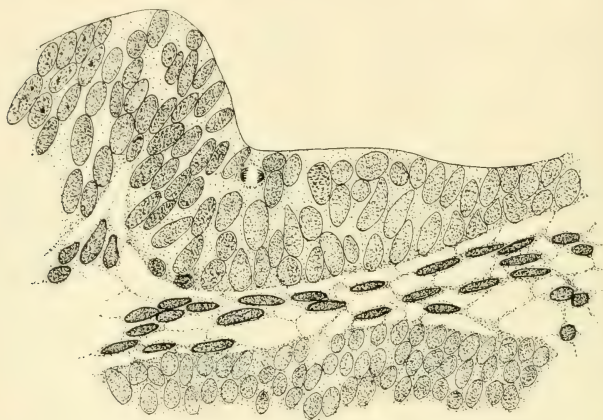


Fig. 2 Portion of the regenerated curtain of an embryo seven days after the same operation as in figure 1, showing regeneration from the margin of the diencephalon. $\times 50$.

composed of only a single layer of cells whose somewhat elongated nuclei are parallel to the surface.

It is evident from the above that the regenerated portion is not made up of nerve cells but rather of the primitive germinal epithelium which lines the neural tube, the only regeneration that takes place being that necessary to close the wound made by the removal of the hemisphere. We have then, forming as a result of the operation, a curtain of primary ependyma across the interventricular foramen. This is not nervous and hence constitutes no true regeneration of the telencephalon.

Hardesty ('04) has shown that the dividing elements of the primary ependyma, the germinal cells, give rise to a nuclear layer just outside of the ependyma from which later develop the neuroblasts and the spongioblasts. It is conceivable that the differentiation of the nuclear layer into nerve cells results from the stimulus derived from the ingrowth of nerve fibers



Fig. 3 Transverse section of embryo without the right hemisphere or nasal placode three months after the operation, showing the thin curtain across the interventricular foramen. $\times 50$.

from other definitive parts of the nervous system. Evidence of this is seen in the fact that the fiber tracts of the telencephalon do not develop until the olfactory nerve fibers have grown into the peripheral part of the hemisphere from the nasal placode. The possibility of such a stimulus has been used by Kappers ('14) in his neuro-biotaxis theory of the phylogenetic migration of nuclear centers in the central nervous system.

On the other hand Harrison ('10) has shown that embryonic nerve cells taken from the medullary cord of frog larvae will produce protoplasmic nerve processes when cultivated in vitro, but it is manifestly impossible to determine by such methods the extent to which development will proceed without the intervention of functional activity. As has been shown elsewhere (Burr '16), development and differentiation of the central nervous system will progress to a certain point without the presence of a functioning end organ. Beyond this point functional activity is essential for continued growth. In the experiments just referred to, the telencephalon was quite completely organized without the ingrowth of the olfactory fibers, but this nerve is not the only one whose fibers penetrate the hemisphere, for Herriek ('10) has shown that in the posterior third of the telencephalon of *Amblystoma* there are at least two centers that are directly connected with the hypothalamus and the pars dorsalis thalami by ascending projection fibers. Hence it seems probable that the apparent self-differentiation of the telencephalon may be due in some degree to the presence of the forward growing nerve fibers which establish this connection, the functional stimulus imparted by them being sufficient to carry differentiation some distance. Experimental evidence that is in favor of this view is seen in the fact that in the posterior margin of the curtain which develops across the foramen of Monro on the removal of the telencephalon and nasal placode, there appears in the older larvae a rounded mass of nerve cells and fibers. This mass is in direct communication with the nucleus habenulae and the hypothalamus, a tract of fibers reaching it from each of these regions. Evidently the neuroblasts at the posterior margin of the interventricular foramen have been stimulated to further development by the ingrowth of axones from lower centers. This mass is, then, in all probability the rudiment of the primitive pallium.

The second series of experiments involved the removal of the right hemisphere without the removal of the nasal placode. The results here are definite and conclusive. A curtain of ependymal cells is formed across the foramen of Monro as in the

previous experiment. The healing of the wound brings the nasal placode back to approximately its normal position though a slight shifting anteriorly has been noted. Five days after the operation the curtain is composed of a single layer of cells (fig. 1). Two days later this single layer of cells has become converted into a thickened mass of columnar cells that are evidently of ependymal origin (fig. 2). By the time the twelfth day is reached the olfactory nerve has established a connection with the curtain, which has in the mean time increased considerably in size. The establishment of the nervous connection between the nasal placode and the telencephalon takes place in the normal larva about the tenth day, only a slight delay in this union occurring as a result of the operation. In the unoperated larvae the nasal placode lies in close apposition to the thick wall of the telencephalon, the olfactory fibers growing into the hemisphere at the point of contact. In the operated forms the nasal anlage lies close to the newly formed curtain, which as indicated above shows a slight thickening not seen in the operated larvae possessing no nasal placode. This thickening anticipates the ingrowth of the olfactory nerve by several days. It is not possible at this time to give any explanation of this thickening, though it is conceivable that the presence of the olfactory anlage stimulates the ependymal cells to grow and divide through some mechanical or chemical factor.

Two days after the ingrowth of the olfactory nerve the new telencephalon, as it must now be called, has reached quite an advanced stage of development. During these two days the thickened curtain has been differentiated into a small but nevertheless typical telencephalon. Neuroblasts are present and a ventricle has appeared. Outwardly the form has changed. A forward growth has converted the thick flat plate of cells into an ovoid mass of neuroblasts and spongioblasts. The ingrowth of the olfactory nerve on the lateral aspect of the mass is accompanied by the appearance of the large lateral forebrain tract (fig. 4). Almost simultaneously there appears from the dorsal aspect of the olfactory bulb the tractus olfactorius dorso-lateralis and from the ventral aspect the tractus olfactorius

medialis. At this time the new telencephalon is similar in organization to its fellow but it is considerably smaller in size. From this time on growth and differentiation continue in both hemispheres in an entirely normal fashion, though apparently there is to some extent a compensating increase in the rate of growth of the new telencephalon for in the oldest larva of the

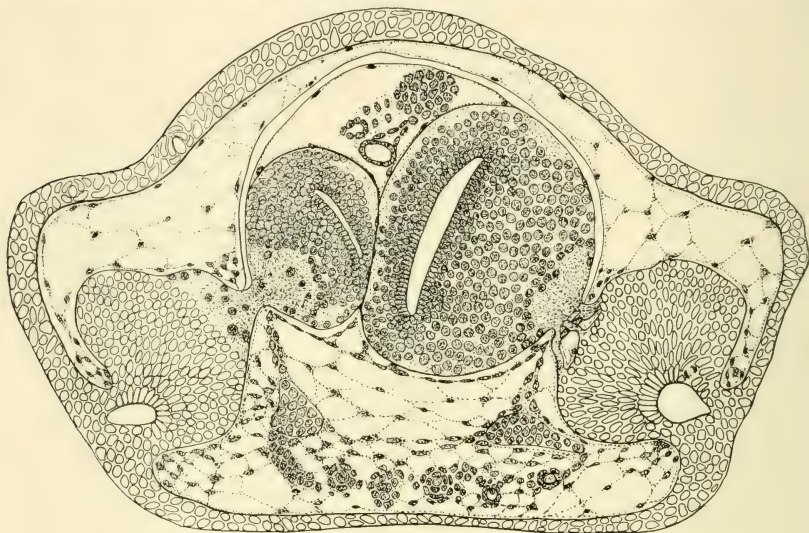


Fig. 4 Transverse section of an embryo one and one-half months after the operation showing the regenerating telencephalon as a result of the presence of the nasal placode. $\times 44$.

series, a larva some three and a half months old, the new hemisphere had completely regenerated and could not be in any way differentiated from its fellow.

SUMMARY

It is evident from the above experiments that the forebrain of *Amblystoma* will not regenerate when it and its functional end organ are completely extirpated. The healing of the wound

results in the formation of a curtain across the interventricular foramen derived from the ependyma lining the neural tube. If, on the other hand, the forebrain is removed without removing the end organ, the nasal placode, the presence of the latter acts as a stimulus to the regeneration of a new telencephalon through the ingrowth of the olfactory nerve. The pallial region of the telencephalon is regenerated in all cases owing to the stimulus afforded by the forward growth of axones from lower centers in the brain and cord.

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THE FUNCTION OF THE EFFERENT FIBERS OF THE OPTIC NERVE OF FISHES

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TWELVE FIGURES (TWO PLATES)

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PRELIMINARY

A number of observations have been recorded, chiefly upon the frog, which indicate that the retinal pigment and visual cells are at least partially under the control of the central nervous system, although the manner and extent of this influence are not well understood. An interrelation between the retinal elements of the two eyes has also been maintained by which stimulating agents, such as light or salt crystals, applied to one retina induce changes¹ in the other. It has not only been declared by

¹ Extensive experimentation has demonstrated the existence of photomechanical responses in the retinal pigment of most of the lower vertebrates. In all cases in which movements of the retinal pigment are demonstrable, light causes an expansion (i.e. a migration toward the external limiting membrane), and darkness a contraction of the pigment (figs. 1 and 4). A portion of the cone's inner member, to which the appropriate term 'myoid' has been applied, is also capable of actively shortening when the retina is stimulated by light, a compensatory

some workers that this interrelation is independent of the brain, but also that it continues after the optic nerve is cut distal to the chiasma. The latter assertion, however, has been the subject of much controversy.

Observations on the frog relative to these conditions have been presented by the following workers: Englemann ('85), Grijns ('91); Nahmmacher ('93); Angelucci ('90; '05); Lodato e Pirrone ('01); Chiarini ('04) and Herzog ('05). Similar statements were made by Pergens ('96) for fishes, and by Birch-Hirschfeld ('06) for the pigeon. The diversity of stimuli which have been reported as being effective in producing positional changes in the cones and pigment has caused Fick ('89; '90; '91) to doubt the reflex nature of the process, although some of his own experiments by no means disprove many of the contentions of the other investigators. More recently Fujita ('11) has shown wherein the older experimentation on the interdependence in the responses of the elements of the two eyes through the action of light, is not trustworthy.

Englemann ('85), who was the pioneer in asserting the presence of 'retino-motor' nerve fibers, further supported his view with results obtained by illuminating the skin only of dark-adapted frogs, whence changes in the cone cells and retinal pigment were said to occur. This observation, however, is not in agreement with those of Fujita ('11) and myself (Arej, '16).

A number of other statements are on record which ascribe a control over the movements of these elements to the central nervous system. An enumeration of stimulating agents supposed to act in this way would include such as the following: noises, unilateral pressure on the eyeball, mechanical irritation (Angelucci, '90), and trussing up a frog for 24 hours (Herzog, '05).

The first direct experimentation in which the relation of the optic nerve to movements of the retinal elements was tested

elongation taking place when the retina is again subjected to darkness (figs. 10 and 11). In fishes and birds, at least, the rod myoid is likewise contractile (figs. 10 and 11), although the direction of movement in light and in darkness is the exact reverse of that executed by the cones. For a review of the literature on this question, reference may be made to an earlier paper by the writer (Arej, '15).

was performed by Hamburger ('89), whose results were corroborated by Fick ('91). After the optic chiasma of the frog was severed, light- and dark-adaption occurred as in normal animals, hence the pigment and cone cells were viewed as independent structures, responding to direct stimulation, the movements of which are not dependent upon the integrity of the optic nerve. Areoleo ('90) likewise found that the retinal elements of the pithed toad exhibited photomechanical changes. On the other hand, Nahmnaehner ('93) showed that stimulation of the frog's optic chiasma with salt crystals induced changes in the cones, only if the optic nerve was intact.

Hence it seems probable that, in the frog, the retinal elements are capable of more or less independent movement, but over this is superimposed a nervous (efferent) control, the nature of which is not altogether evident.

Apart from the work of Pergens ('96), who believed that the illumination of one eye of a fish induced cone contraction in the other, practically no attempt has been made to determine the conditions under which movements of the retinal elements of fishes are accomplished. The negative results of Gertz ('11) concerning the effect of electrical stimulation of the eyes of *Abramis brama* differ only slightly from those of Fujita ('11), who, however, maintained that induction shocks caused an insignificant pigment contraction in the light-adapted eye of the 'Weissfisch.'

STATEMENT OF THE PROBLEM

It has been shown that the retinal pigment of most fishes expands to a marked degree when the normal animal is brought from a situation of darkness to one of light, and that the pigment again contracts when the procedure is reversed (figs. 1 and 4).

The writer (Arey, '16) has also found that the pigment in the excised eyes of *Ameiurus*, at least, is capable of expanding when subjected to light, which, in this case, presumably acts as a direct stimulus on the pigment cells; a contraction in darkness, how-

ever, does not occur. In several other fishes (Abramis; Fundulus; Carassius) a general pigment migration could not be demonstrated under these conditions even in the light, and such a lack of response was accounted for by postulating the occurrence of an autoanaesthetization of the pigment cells, presumably through the accumulation of catabolic wastes. Furthermore, the rods of the excised eyes of Ameiurus undergo movements both in the light and in the dark, whereas the cones move in the light only; yet neither darkness nor light induces positional changes of the cones in the excised eyes of certain other fishes (Abramis and Fundulus). As will be seen farther on, the total absence of response in the excised eyes of these fishes throws important light upon the question of a probable autoanaesthetization of the retinal elements.

If the optic nerve transmits afferent impulses exclusively, as hitherto has been believed, no disturbance in the movement of the retinal pigment should be introduced when the optic nerve only is severed, and the retina is thereby freed from this source of cranial innervation. The present paper will be devoted to the description of experiments devised to test the validity of this hypothesis.

In order that the reader may better interpret the various experiments about to be described, as well as the significance of their progressive sequence, it is advisable to state in advance the thesis which this paper aims to establish. Experimental evidence will be presented which offers physiological proof for the existence in Ameiurus of two distinct components of a mechanism, through the balanced action of which are movements of the visual cells and retinal pigment alone possible. One component involves the efferent nerve fibers of the optic nerve, whereas the second component (possibly the ciliary, autonomic, nerves) is closely associated with the eye muscles. This latter set of nerve fibers exerts a constant inhibition upon the movements of the retinal elements, while the impulses in the efferent optic nerve fibers, on the other hand, serve only as a block to this tonic inhibition, thus allowing photomechanical responses to occur.

The following paper presents one phase of an investigation upon the visual cells and retinal pigment, pursued at Harvard University under the supervision of Prof. G. H. Parker. To Professor Parker I am greatly indebted for the continued interest and kindly criticism that characterizes his instruction.

MATERIAL AND TECHNICAL METHODS

The fishes used in this investigation were as follows: the common horned pout, *Ameiurus nebulosus* Lesueur; the shiner, *Abramis crysoleucas* Mitchill; and the common killifish, *Fundulus heteroclitus* Linn. A greater part of the experimentation was done upon *Ameiurus*, since in this animal the activities of the retinal pigment and visual cells proved to be especially favorable under the experimental conditions imposed upon them. Furthermore, the occurrence of responses in the excised eyes of *Ameiurus* was a happy concomitant circumstance, since the results were thereby controlled at several important points.

The technical methods used in preparing retinas for microscopical examination were simple. The eyes of *Ameiurus* were excised directly, for the skin of this animal is soft and the eyes are prominent. In the two other fishes, especially when rapidity of operation was desirable, the following procedure was observed. With heavy scissors the cranium was bisected in the sagittal plane; following this, a transverse cut just posterior to the orbit freed the two halves of the cranium, with the contained eyes, from the rest of the body. In either case the operation was performed in a few seconds, and the eye, without being handled, was allowed to drop into the fixing fluid.

Perenyi's fluid gave good fixation and was used exclusively. Fixatives containing nitric acid have long been recognized as favorable in the obtainment of faithful preservation of the retina. The preparatory steps prior to embedding in paraffine demand generous allowances of time, yet the processes of dehydration and clearing should progress as rapidly as possible, since otherwise the sclera becomes extremely hard.

Two methods were used in removing the lens, one of which, although longer, gave much more satisfactory results. The first,

somewhat tedious, procedure consisted in paring away the front face of the eyeball with a razor after the eye had previously been imbedded in paraffine. After removing the face of the eyeball slightly beyond the ora serrata, the lens was pried from its paraffine matrix with a dissecting needle; following such manipulation reimbedding was of course necessary. The second and simpler method was to remove the face of the eyeball with fine curved scissors after the eye had been hardened in absolute alcohol; unless, however, the eye was sufficiently hardened and the greatest care exercised, the retina proper easily separated from the pigmented epithelium. On the whole, the first method was preferred to the second because of the wrinkling of the retina that usually accompanied the use of the latter.

Sections were cut 7μ to 10μ thick, and only those passing through the region of the optic nerve were retained for examination. Preparations were stained with Ehrlich-Biondi's triple stain or were double stained in Heidenhain's iron haematoxylin and a plasma counterstain. Ehrlich-Biondi in some instances gave excellent differentiation of all elements, while at other times it would show the capriciousness for which it is notorious: iron haematoxylin gave uniformly good preparations.

When it became necessary to bleach the pigment, in order to study the visual cells, which would otherwise be masked by the partially or completely extended processes, the method employed was essentially that of Mayer, in which nascent oxygen² is the effective agent.

EXPERIMENTAL PART

a. Experimentation upon Ameiurus

Ameiurus is well adapted for operations involving the optic nerve. The soft skin and the relatively small eye allow easy access to the orbit without causing the serious disturbance and shock that is almost unavoidable when operating on fishes which have eyes set in prominent bony sockets.

² When potassium chlorate and hydrochloric acid interact, it is commonly said that nascent chlorine is the agent causing bleaching. As a matter of fact the reaction liberates free oxygen.

The method of operation was as follows. With curved scissors an incision was made in the skin ventral to the eye and the cut carried upward around both sides of the ball until a semicircular (or greater) incision resulted. The eyeball could now be rolled back, and when thus displaced, no excessive strain was exerted on the conjoined tissues. By dissecting through the aperture thus exposed, the optic nerve was separated from the overlying muscles and severed. If a delicate razor-edge scalpel, such as ophthalmologists use in cataract operations, is employed, it is comparatively easy to cut the nerve without injury to the surrounding parts. *Ameiurus* has no large central artery and vein in the optic nerve, as is the case in mammals. A blood-vessel, however, runs beside the optic nerve, but if care is taken it need not be injured by the operation.

1. *Retinal pigment.* My first effort was to discover the effects upon pigment migration of the severance of the optic nerve only. A typical experiment will illustrate the response of the pigment when a previously dark-adapted fish was operated on and exposed to light.

Experiment 8.1.12. The optic nerve of a dark-adapted fish was severed and the animal allowed to recover from any shock effect until the next day, when it was exposed to diffuse daylight for $2\frac{1}{2}$ hours. At the expiration of this time both the operated and the normal eye were excised. Subsequent examination showed that the retinal pigment in the operated eye had retained the position typical of darkness (fig. 4), while the pigment in the control eye had migrated normally (fig. 1).

Thus it is seen that the integrity of the optic nerve must be maintained in order that the retinal pigment of an otherwise intact animal may undergo a positional change when stimulated by light.

In successful experiments of this type the length of exposure varied between 45 minutes and 3 hours, yet uniform results were obtained.³ A possible influence of operative shock, on animals that were subjected to experimentation directly after the

³ Under normal conditions the pigment is fully extended by light in about 45 minutes (Arey, '16).

optic nerve was severed, was shown to be non-existent. It was also shown that roughly performed operations, in which various mutilations of the eye muscles and the adjacent blood vessels were intentionally made, did not interfere with the obtainment of essentially typical results.

This type of experiment has been repeated very many times. Among animals used during both the spring and the fall of 1913, no deviation was found from the conditions recorded above. When work was begun again on a new supply of *Ameiurus* in the spring of 1914, the results secured from operated animals brought from darkness into the light, were not always as decisive as those of the preceding year. Some experiments showed a complete retention of the pigment, while others resulted in a partial migration, which, in some cases, was quite extensive although never as complete as in normal light adaption. Further experimentation on other animals procured in the fall of 1914 also gave inconsistent results, varying from complete to very incomplete control.

I am unable to state the cause of these discrepancies. That the results of the work done in the year 1913, which embraced the larger part of this experimentation, are beyond question, I feel confident; the remarkable consistency of a long series of experiments about to be described substantiates this conviction. On the other hand, the persistent occurrence of more or less incomplete, mingled with complete control in the work of the year 1914, was also undeniable.

It is possible that individual fish vary considerably in their ability to inhibit the migration of pigment after sectioning of the optic nerve, or what amounts to the same thing, the activity of the pigment in producing expansion may be variable. I shall attempt to present evidence, based on experimentation to be described later, that demonstrates an inhibiting mechanism which prevents the pigment from migrating when the optic nerve is severed. Since it has been proven (Arey, '16) that the action of light on completely excised eyes can directly stimulate the retinal pigment to movement, it is evident that there must be a competition between light stimulation and this inhibiting mech-

anism for the control of pigment migration. If the inhibiting mechanism is weak or becomes exhausted, or if the response of the pigment cells to the direct action of light is especially strong, the common result will be the production of a more or less extensive movement of the pigment.⁴

This, nevertheless, does not explain how different lots of animals, obtained in the spring and fall of one year, differed as a whole from other lots, procured in corresponding seasons of the following year. All the fish used were supplied by one collector, who secured them from several small ponds, according to their varying abundance from year to year. It is possible that the stocks of different ponds may show more or less consistency, due to common inheritance, in the behavior of their retinal pigment under the previously mentioned experimental conditions. The writer, however, wishes to present this suggestion merely as a possibility that may or may not be true.

A second type of experiment, in which light-adapted animals were subjected to darkness, is illustrated by the following record.

Experiment 8.1.49. One optic nerve only of a light-adapted *Ameiurus* was severed. The animal was subjected to darkness for 18 hours, after which both eyes were excised. No pigment contraction was found to have occurred in the operated eye (fig. 2), whereas the pigment of the control eye showed the extreme contraction of dark-adapted retinas (fig. 4).

The greatest amount of contraction ever observed was a withdrawal of the distal accumulation of pigment, which even at room temperature is often characteristic of light adaption, to form a uniform zone (fig. 2). Sometimes a slight thinning out of pigment was seen, whereby the expanded mass appeared less dense than normally. In no case, however, was there observed a withdrawal of pigment, as a whole, from the zone usually

⁴ A few determinations of the rapidity of pigment adaption seemed to indicate that the migration occurred more slowly in the fishes used during the first season, which gave constant results, than in the animals used during the following year. This fact not only tends to support the hypothesis that a variability in the activity of pigment migration was responsible for the lack of consistency in the later results, but also indicates that the response of the pigment in the animals of the first year, taken as a whole, may have been less vigorous.

occupied during light adaption. In this type of experiment, therefore, it is also evident that severance of the optic nerve restrains the normal pigment migration. The fact that the pigment of excised eyes does not contract in darkness, and that pigment contraction in general seems to involve a less vigorous response than does expansion, explains why consistent results were obtained in all these experiments, for the competition with the inhibiting mechanism presumably was less keen.

To remove any doubt that the integrity of the optic nerve is necessary for the migration of pigment in light and in darkness, the nerve was cut inside the cranium near the chiasma. In order to do this, a small aperture was made in the cranium over the region of the optic nerve and the severance of the nerve was accomplished by introducing a cataract scalpel through the opening thus made. The removal of bone from the thick cranium had to be done with care or the fish failed to recover from the resulting shock effect. In successful experiments the results obtained were similar to those previously recorded. Since the eye and the surrounding parts were left intact during the whole procedure, it is safe to conclude that in *Ameiurus* the optic nerve is intimately related to the phenomenon of pigment migration.

A microscopical study of preparations demonstrating the relation of the optic nerve to the nerve-fiber layer of the retina shows that the arrangement of nerve fibers is approximately of a radial nature, and that the fibers from any sector of the retina are extended into the adjacent side of the optic nerve. These relations probably become disturbed a short distance from the emergence of the optic nerve from the eyeball. The optic nerve of *Ameiurus* joins the retina by several roots (fig. 7, *rdx. n. opt.*), hence this condition is not shown as diagrammatically as in preparations of the eye of *Abramis* which cut the optic nerve in a radial plane. Here the nerve fibers form a distinct V-shaped 'parting' as they pass to the two sides of the retina. On the periphery of the nerve this is especially evident although at the center there is undoubtedly more decussation, as was shown by Kohl ('92) for several of the lower vertebrates.

This relation should stand the physiological test imposed by partial section of the optic nerve. In view of the results already established, one would expect to find the pigment situated in a sector adjacent to the cut to be unaffected by varying conditions of light and darkness, while the pigment adjacent to the intact portion of the nerve should expand or contract in a normal manner. The description of an actual experiment will make this clear.

Experiment 8.1.38. The optic nerve of a light-adapted *Ameiurus* was two-thirds severed close to the eyeball, and the fish was transferred to darkness. After 2 hours the eye was excised, a pointed flap of skin being left attached to the eyeball to insure proper orientation. In a preparation, sectioned to include portions of the retina adjacent to both the cut and the uncut fibers, a striking contrast was evident (fig. 7). On the uncut side the pigment was contracted maximally, while on the cut side it still remained in the expanded position characteristic of light adaption.

I can think of no experiment that could be devised to support better the view concerning the rôle which the optic nerve plays in the movement of retinal pigment, than the one just described.

The similar treatment of dark-adapted fish which were subjected to light did not give as decisive results as those of the reciprocal set. The pigment on the 'cut' side of the eye was not retained in a contracted state, but migrated to a considerable extent (fig. 6). In no case, however, was the expansion maximal, that is, involving an accumulation near the external limiting membrane, but at most only a broad evenly pigmented zone was formed, which was markedly in contrast with the maximal expansion of pigment in that half of the retina adjacent to the intact portion of the nerve.

By the evidence of previous experimentation, a reason for this difference in behavior is suggested. It has already been shown that light is an efficient stimulus in producing expansion of the pigment in excised eyes, whereas darkness does not contract expanded pigment. Furthermore, after complete section of the optic nerve, the pigment in certain cases still tended to migrate when the animal was exposed to light. In the experiment last described, it is probable that there was a more or less extensive

decussation of fibers distal to the cut, and that these fibers, although few in number, since they come from the intact portion of the nerve and distribute themselves through the retina of the opposite side are capable of allowing the pigment to migrate when the eye is subjected to the action of light. In producing this result, these fibers are doubtless servient to the direct action of light on the pigment cells themselves. In darkness, without the aid of some independent and efficient factor, such as the direct stimulus of light, it may well be that these stray fibers are not sufficiently potent to overcome the inertia of the pigment.

If the cause of the apparently inconsistent behavior described in these two types of experiment is something of the nature of that just outlined, then the evidence for the control of pigment migration through the optic nerve thereby receives additional support.

It was thought that if a small cut were made through the eyeball and retina, near the entrance of the optic nerve, the portion of the retina peripheral to the cut would be freed from all connections with the optic nerve, and consequently some decision could be reached concerning the rôle of the decussating fibers just considered. This method, if successful, would also corroborate the general conclusions which were drawn as to the control of pigment migration by the optic nerve fibers.

On the whole, the results showed quite conclusively that in the earlier experiments the presence of decussating fibers had caused the pigment expansion on the side of the retina adjacent to the cut optic nerve, when the eye was stimulated with light. Cuts about 1.0 mm. in length were made with a cataract scalpel. Although the blade of the scalpel was very thin, tapering to a needle-like point and the edge was of great keenness, nevertheless, in many cases the retina was torn away from the contracted pigment layer. The ease with which a separation of the retina and the pigment layer is effected, especially when the pigment is not expanded to strengthen the union, is well known.

In successful operations (fig. 5), the pigment lying peripheral to the cut showed but little expansion, although it was often the case that toward the extreme periphery of the retina, pigment

expansion was again found. Since it is probable that the fibers of the nerve-fiber layer do not have a precise radial distribution, the latter condition is readily explained by assuming the presence in this peripheral expanded region of intact nerve fibers, which, near the fundus, bordered on the incision.

The decrease in intraocular pressure necessitated by cutting the eyeball has no effect on the activity of the pigment, for normal animals whose corneas have been punctured show typical responses.

One is driven to the conclusion, by all the experiments heretofore described, that there must be some mechanism, either in the eye muscles or in the blood vessels of the eye, that exerts an inhibition on the movements of the retinal pigment, for proper control experiments have eliminated the skin as a possible factor.

If this supposition is true, the retinal pigment should undergo expansion when all the eye muscles and blood vessels of a dark-adapted fish are cut, and the eye, connected to the body by the optic nerve only, is subjected to light. This condition was indeed realized, the pigment distribution being essentially like that in totally excised eyes. The same experiment under reversed light conditions did not result in a contraction of the pigment. As in the similar failure of excised eyes to show contracted pigment when subjected to darkness, I believe there is a strong probability of an anaesthetic action on the pigment cells due to the accumulation of catabolic waste. If the vascular circulation could be preserved, it is probable that the pigment cells would contract in an experiment of this kind. Possibly with artificial circulation the pigment of an excised eye would also contract in the dark.

The next step was to discover whether the presence of certain eye muscles could be correlated with the inhibition of the pigment response. When dark-adapted *Ameiurus*, having the optic nerves cut, were brought into the light and the dorsal oblique and posterior rectus muscles (those innervated by trochlear and abducens nerves respectively) were severed, no pigment migration occurred. It is evident, therefore, that the inhibiting mechanism does not involve these muscles alone. Reciprocally, the dorsal, ventral

and anterior rectus and the ventral oblique muscles (those innervated by the oculomotor nerve) were cut, leaving the eyeball attached to the body by the two remaining muscles. Under these conditions, the pigment migrated much as in a normal animal.

Hence it appears that the inhibitory mechanism has been located as existing in association with the muscles (or possibly the blood vessels of the muscles) which are innervated by the oculomotor nerve. The objection may be raised, however, that there is a possibility of the dorsal oblique and posterior rectus muscles possessing an inhibitory function also, but that the inhibition produced when only two muscles are left in connection with the eye is not sufficient to prevent the pigment response. The evident check experiment which answers this criticism consists in severing all the muscles except two which are innervated by the oculomotor nerve. When this was done (the dorsal rectus and the inferior oblique being left intact) no movement of the pigment was observed. This result indicates that the inhibitory mechanism is found associated with those muscles innervated by the oculomotor nerve and is not demonstrably associated with the other eye muscles.

Since the dorsal oblique and posterior rectus muscles are not potent in restraining pigment migration, it was possible to make the following experiment. All the muscles excepting these two were cut and light-adapted fish with the optic nerves either intact or severed were placed in a dark situation. If the retinal pigment contracted under these conditions, it could be reasonably assumed that the presence of a partial vascular circulation had been responsible for the change. However, no movement of the pigment was detected, yet as the blood vessels in these muscles are small in number and size, the results neither support nor detract from the general view that absence of blood supply prevents pigment contraction in the dark.

Although the relation of the autonomic fibers to the oculomotor nerve of teleosts has not been worked out as completely as in some other forms, it is at least evident that from the ciliary ganglion the so-called ciliary nerves pass to the eyeball, probably

following the same general courses as do the branches of the oculomotor nerve. In man, where the ciliary nerves have been traced rather carefully, autonomic fibers supply the sclerotic and choroid coats, ciliary muscle, iris, and cornea of the eyeball (Carpenter, '06). The ciliary ganglion of fishes according to Önni ('01) also receives fibers from the trigeminal nerve, the relation being more intimate than Schwalbe ('79) believed.

The action of atropine upon sympathetic nerves is to paralyze the endings of the postganglionic fibers. If the mechanism that inhibits pigment migration involves sympathetic nerves, it was thought that it might be possible to eliminate its action by the use of this drug.

By supplying fishes with water through a tube, they could be retained indefinitely in the air. Dark-adapted fish with severed optic nerves were brought into the light, and occasionally during the course of an hour small amounts of a 0.5 per cent solution of atropine sulphate were introduced into the orbit. Subsequent examination of the retina showed that a migration of pigment to the light phase had occurred.

The possibility of a direct stimulation of the pigment cells presented a difficulty, however, that had to be tested, for Spaeth ('13) found that a 1.0 per cent solution of atropine caused a rapid expansion of the melanophores of *Fundulus*. When dark-adapted eyes were placed in a 0.5 or 1.0 per cent solution of the drug and were returned to darkness for an hour or more, the pigment did expand to an extent which nearly equalled that caused by incomplete light adaption.

It is certain, therefore, that any evidence gained from experimentation of the kind described is valueless, and it is doubtful if much more dependence can be placed on the significance of a pigment migration when atropine was painted on to the eye muscles in such limited amounts that a direct stimulation of the pigment cells seemed improbable, or when small quantities of atropine were injected into the cranial cavity.⁵

⁵ The use of nicotine, which paralyzes the synapse of the preganglionic fiber with the sympathetic nerve cell, might furnish more interesting results, provided it did not have a toxic effect on the pigment cells. Unfortunately, no experiments of this kind were performed.

An attempt was made to cut the oculomotor nerve and thus to separate the sympathetic fibers which arise with it from the brain. To make a large opening through the cranium leads to operative shock from which *Ameiurus* does not recover. Accordingly, a relatively small aperture was made, through which a cataract scalpel was introduced, all the nerves presumably being cut on one side of the brain between the optic and the trigeminal. A dark-adapted *Ameiurus* with severed optic nerve, when exposed to the light after this treatment, showed pigment expansion. This experiment, although properly controlled, was not of a refined type. It is possible that disturbances other than the mere section of the oculomotor nerve may have led to the observed results.

It is not intended that any of the experimentation in which an attempt was made to locate the fibers of the inhibiting mechanism, should be received as conclusive. The results obtained from cutting muscles and from the severance of the oculomotor nerve suggest that autonomic fibers are involved, and that these enter with the oculomotor nerve. A strong suspicion is therefore cast upon the sympathetic fibers in connection with the ciliary ganglion as the causal agents in preventing movement of the retinal pigment when the optic nerve is cut.

No statement has previously been made concerning the nature of the mechanism in the optic nerve, the integrity of which is necessary for allowing positional changes of the pigment to occur. If the inhibitory fibers associated with the eye muscles are conceived as acting after the manner of a brake, it follows that the optic nerve must contain active components, which in some way, directly or indirectly, permit expansion and contraction of the pigment.

If the optic nerve contains fibers of an efferent nature, it is of interest to discover whether these components can be made to function by electrical stimulation. The description of a typical experiment will best illustrate this point.

Experiment 8.1.23. A previously dark-adapted *Ameiurus*, in which both optic nerves had been cut, was retained in the air, respiratory water being supplied through a tube. The peripheral end of the cut

optic nerve of one eye was exposed and stimulated for $1\frac{1}{4}$ hours, in the light, with a weak current from an inductorium. Inspection of the two retinas showed that the pigment in the eye that had been electrically stimulated was uniformly expanded (fig. 2), while in the control eye the pigment still remained contracted as is characteristic of dark adaption (fig. 3).

Since repeated trials confirmed these results, the conclusion follows that there must be nervous elements of an efferent nature in the optic nerve of this fish which normally receive impulses from the central nervous system, and can be made to function experimentally by electrical stimulation. The result in either case is a release from an inhibition exerted upon the pigment cells by a mechanism possibly involving the autonomic fibers from the ciliary ganglion.

Previous experimentation (Arey, '16) has shown that both in light and in darkness the retinal pigment of fishes is more highly expanded at low temperature ($0^{\circ}\text{C.} +$) than at high temperatures ($25^{\circ}\text{C.} \pm$), hence a series of experiments was next made to discover whether after the optic nerve was cut temperature would still be efficient in producing the characteristic temperature responses. In determinations made both in light and in darkness, the results were identical with those found in normal animals, for at 5°C. , the expansion of pigment was greater than at 25°C. (figs. 1 and 2; 3 and 4).

In order to produce sharp contrasts (in the light at least), before the optic nerve was cut, a preliminary treatment at a temperature of 5°C. was necessary. If the preliminary treatment was at 25°C. the pigment remained in the position characteristic of that temperature, regardless of the temperature that followed. From this result additional evidence is obtained, over that already advanced (Arey, '16), to show that a high temperature is more efficient in causing positional changes of the retinal pigment than is a low temperature, and that temperature is more efficient than either light or darkness.⁶

⁶ It should be remembered, however, that temperature merely produces a quantitative redistribution of the already expanded or contracted pigment, the extent of its influence always being limited in this way.

2. *Visual cells.* The more important experiments, which show the relation of the optic nerve to the pigment migration of *Ameiurus*, were repeated in order to discover whether a similar set of relations exists between the optic nerve and the visual cells.

It will be remembered that the myoid of the cone cells of fishes elongates in the dark and shortens in the light (figs. 10 and 11), whereas the behavior of the rod myoid is the converse of this. Moreover, in excised eyes both types of cells are stimulated to movement through the direct action of light, whereas only the rods show a response in darkness.

In general it may be said that these experiments, which extended discontinuously throughout a period of two years, showed that whenever the migration of pigment was inhibited the movements of the rods and cones likewise failed to occur. In those cases, discussed previously (p. 220), where cutting of the optic nerve only partially inhibited the pigment response, the movements of the visual cells usually were not prevented. Practically all of the experiments to be described were performed in the spring and autumn of 1913 before cases of incomplete control were discovered.

When the optic nerve of a dark-adapted fish was severed, and the animal was subjected to light (fig. 12), the rods remained as in the condition of dark adaption. The cones, on the contrary, usually became more or less shortened, although they did not approach the external limiting membrane as closely as when exposed to light under normal conditions.

In the converse experiment, in which operated fish were transferred from light to darkness, the cones showed no tendency to elongate, but in some cases remained maximally shortened for several hours. The rods, however, did not always retain the highly elongated position characteristic of darkness, for they commonly exhibited partial retraction, although the final position was unmistakably that of semi-elongation.

From these two types of experiment one striking correlation is evident. The cones have a greater tendency to undergo positional changes in the light, while the rods behave similarly in

darkness. The end result of this tendency towards movement, nevertheless, is identical in both kinds of visual cells, for a shortening of the myoid results in either case. This would suggest that the process of retraction, as in simple contractile tissue in general, is more vigorous than is elongation. This behavior of the cones is comparable to that observed in excised eyes of *Ameiurus*, where these cells shortened in the light but remained unchanged in darkness; the rods of excised eyes, on the contrary, moved both in light and in darkness. The posulation of a more vigorous response in retraction offers an explanation for the observed facts, for under such conditions the inhibitory mechanism would be less likely to check completely the positional changes of these elements.

Reference has been made (p. 223) to experiments in which the optic nerves of light-adapted fish were partly severed. When these animals were transferred into the dark, the retinal pigment showed distinct areas of expansion and contraction, corresponding respectively to the cut and uncut sides of the optic nerve. In one such preparation which had been stained, the position of the cone cells was also observed to vary with respect to the same areas. Where the pigment had undergone movement, the cones were elongated, whereas in the half of the retina adjacent to the cut fibers the cones remained maximally shortened.

A control experiment, in which the light-adapted eye, connected to the body by the optic nerve only, was subjected to darkness, showed that the rod cells had shortened although not always completely. The cones, as in excised eyes, did not change their positions in the least. There is no evidence that the optic nerve of itself prohibits the movements of the rod and cone cells.

The results which have thus far been outlined indicate that, just as with the retinal pigment, there is a mechanism associated with the muscles or blood vessels of the eye which tends to inhibit movements of the visual cells. When the dorsal oblique and posterior rectus muscles are cut, and after section of the optic nerve the dark-adapted *Ameiurus* is exposed to light, no changes in the position of the rods and only incomplete

changes of the cones occur. The muscles innervated by the trochlear and abducens nerves evidently are not essential in causing an inhibition of movement.

A few reciprocal experiments were performed by cutting all the eye muscles except the dorsal oblique and the posterior rectus. Although the extent of positional change was not as striking as in excised eyes, it seems probable that the same mechanism that controls the migration of pigment, and is presumably associated with the muscles innervated by the oculomotor nerve, also acts on the visual cells.

The evidence, therefore, suggests that normally there are impulses which travel in the optic nerve and render ineffective the inhibition produced by a second set of fibers. Hence it becomes a matter of interest to discover whether it is possible, by artificial stimulation of the severed optic nerve, to cause the visual cells to move more freely than they would otherwise do. The details of these experiments were similar to those previously described when the migration of pigment was tested. The cut optic nerves of dark-adapted fish were stimulated in the light by a weak faradic current. Both rod and cone cells assumed their characteristic light positions, while in control eyes of the same animals, the optic nerves of which had been cut but not stimulated, the visual cells remained for the most part as in darkness.

These results on the visual cells of *Ameiurus*, taken as a whole, closely agree with those obtained from the study of retinal pigment, and the general conclusion concerning their significance is so identical with one previously stated (p. 229) that it hardly needs to be repeated here.

It was shown in a former paper (Arey, '16) that in fishes an elevated temperature ($25^{\circ}\text{C}.$ \pm) causes an elongation of the myoids of dark-adapted cone-visual cells, whereas a low temperature ($0^{\circ}\text{C}.$ \pm) induces a shortening of the myoids. The rod myoid exhibits a similar behavior but in a less marked degree.

The effect of temperature was tested on the visual cells of dark-adapted fish the optic nerves of which had been cut. At $5^{\circ}\text{C}.$ in the dark the cone myoid measured $18\ \mu$, the rod myoid $8\ \mu \pm$ (fig. 8). At $25^{\circ}\text{C}.$ in the dark the cone myoid elongated to $25\ \mu$, the rod to $10\ \mu \pm$ (fig. 9). These results are in agreement with

those obtained with normal animals. The effective action of high temperature in causing an elongation of the cone cells is noteworthy, since without the aid of temperature no change in the position of the cones occurred under these conditions.

b. Experimentation upon Abramis and Fundulus

Having presented evidence which indicates that the optic nerve fibers of *Ameiurus* are not all afferent in function, the question arises as to the occurrence of this condition among other fishes as well as among other classes of vertebrates. A series of experiments was carried out upon *Abramis* and *Fundulus* in which the effect produced on the retinal elements by the severance of the optic nerve was tried.

Both in light and in darkness the retinal pigment and the cone cells of *Abramis* underwent movements which were essentially normal, and hence independent of the cut nerve. Both in the cone myoid, which is capable of a 90 per cent retraction, and in the pigment, which exhibits extreme conditions of contraction and expansion, was an independent movement strikingly exhibited. The stain used in making these preparations did not demonstrate the rods to advantage, except in one instance where a light-adapted fish had been subjected to darkness. In this case the rods were shortened, occupying the characteristic dark position.

When all the eye muscles and blood vessels of dark-adapted *Abramis* were cut, the eye remaining attached to the body by the optic nerve only, no marked change in the position of the pigment or cones accompanied a removal into the light. Since the pigment and cones show no movements when the eyes are completely excised and exposed to light or darkness, these experiments favor the view that the absence of movement in such cases is not due to an inhibition through the optic nerve but to an interruption in the vascular supply.⁷

⁷ In several instances a large blood vessel that lies near the optic nerve was accidentally cut in experiments where the optic nerve only was being severed, yet the changes in the retinal elements occurred as before. This further supports the view that the blood supply to the retina by means of vessels in connection with the eye muscles is important in allowing the positional changes to occur.

The effect of severing the optic nerve of *Fundulus* was investigated in a similar manner. When brought from darkness to light the pigment migrated normally after the usual operation. The converse experiment also showed an independent movement of the pigment, although the poorly defined contraction which characterizes dark adaption rendered this type of experiment less decisive than the clear cut results on *Abramis*.

It is evident, then, that unlike *Ameiurus* the movements of the retinal elements of *Abramis* and *Fundulus* are not dependent upon the integrity of the optic nerve. From the results of Hamburger ('89) and Fick ('91) upon the frog, who showed that the retinal pigment underwent movements after the optic nerve was cut at the chiasma, it is safe to conclude that in this animal also the optic nerve does not control pigment migration. The experiments of these latter workers are all the more interesting since in many observations which have been recorded showing the retinal elements of the frog to be under nervous control, it has generally been directly stated, or at least implied, that the optic nerve was involved. It would be unprofitable to speculate concerning the further occurrence among the vertebrates, or even among the fishes, of individuals possessing efferent optic nerve fibers which act similarly to those in *Ameiurus*.

THEORETICAL CONSIDERATIONS

Although serious doubt has been cast on many of the earlier results upon the frog which were supposed to demonstrate the presence of efferent fibers, both between the brain and the retina and between the two retinas by way of the optic chiasma, yet it is probable that there is a residue of truth in the general proposition of a nervous control of the retinal elements in this animal.

Fick ('90, p. 84), in a paper attacking Englemann's assertion of the existence of efferent nerve fibers, takes the following position:

Wenn dieser Schluss richtig ist, so kann man nur ruhig durch die ganze bisherige Sinnesphysiologie einen Strich machen und ihre Erforschung von Neuem beginnen; denn die rein centripetale und spezifische

Leitung in den Sinnesnerven gilt als die eigentliche Grundlage dieses Abschnittes der Physiologie.

This statement may have been partially justified in view of the diversity of stimuli reported to be potent in causing changes in the retinal elements of the frog, but even if the presence of efferent fibers of the kind physiologically demonstrated in *Ameiurus* were found in all the vertebrate classes, it by no means follows that the fundamental principles of sensory physiology would be seriously endangered. In the normal *Ameiurus* the efferent function does not in the least interfere with the movements of the retinal elements, and stimulating agents which do not act directly upon these cells presumably are ineffectual in producing changes. Since, moreover, there is no evidence of any intrusion on the part of these elements upon any of the sensory processes, our ideas of retinal physiology scarcely require modification and certainly do not demand reorganization.

From the study of *Ameiurus* experimental proof has been advanced showing the existence of an inhibitory mechanism, not associated with the optic nerve, which tends to prevent the movements of pigment cells and retinal pigment. Moreover, a second mechanism associated with the optic nerve was demonstrated, the integrity of which is necessary for the accomplishment of typical movements on the part of the retinal elements.

It may now be fairly asked whether there is any evidence indicating the possible *modus operandi* of these two systems. Several schemes are readily suggested by which the facts hitherto presented could be explained. If, however, one conceives of the efferent fibers in the optic nerve as actively causing movements of the retinal elements, both in darkness and in light, either two kinds of efferent fibers must be postulated, or one movement of each of the elements is passive—a return to the unstimulated condition—and in some way is interfered with by the inhibitory mechanism when the optic nerve is cut. Nevertheless, either one of these explanations becomes discrepant when applied to the total behavior of the retinal elements.

Since electrical stimulation of the cut optic nerve in the light induced changes in the retinal elements of an otherwise intact

fish, it would seem reasonable to expect that the same would occur in darkness, provided efferent impulses in the optic nerve directly stimulate the retinal elements to undergo positional changes.

Gertz ('11) tested the effect of electrical stimulation for one to two minutes in darkness and in light upon the eyes of *Abramis brama*, both excised and in vivo, but only negative results were obtained. Englemann ('85) had previously asserted that the cones and retinal pigment of the excised or normal eyes of dark-adapted frogs responded to induction shocks in a similar way as to light, and Arcoleo ('90) also claimed to have observed pigment migration under these conditions in a pithed toad and frog, as well as upon dark adapted excised eyes. The more recent work of Lederer ('08) and of Fujita ('11) upon normal frogs nevertheless, has not supported this view.

When the cut stump of the optic nerve of a dark-adapted *Ameiurus* was stimulated with a faradic current in total darkness, no distinct changes occurred in the retinal pigment or rod cells, even though the stimulation continued for 45 minutes. Similar treatment of excised eyes also gave negative results. These observations indicate that a satisfactory explanation of the action of the efferent nerve fibers must be sought by viewing the situation in a different way.

It is instructive to adopt the point of view suggested by a study of the vasomotor nerves. In this case a tonic condition is presupposed either through the action of vasoconstrictor nerve fibers or possibly by the intrinsic properties of the muscles themselves. Dilation is believed to be accomplished by the action of dilator nerve fibers, whose impulses inhibit the tonic contraction of the musculature, thus indirectly causing relaxation. To work out a detailed application for the condition found in *Ameiurus* would be both unprofitable and unwarranted. The simplest conception is that impulses⁸ from the efferent compo-

⁸ This view of tonicity differs greatly from that which Herzog ('05) believed to exist in the frog. His statement that after destruction of the central nervous system the pigment and cones gave abnormally vigorous responses in the dark, as if released from an inhibition, does not agree with the observations of Hamburger ('89), Arcoleo ('90), Dittler ('07) and Garten ('07).

nents of the optic nerve block, i.e., counteract, the tonic inhibition produced by the second nervous mechanism, thereby allowing conditions of light and darkness to act directly upon the retinal elements.⁹ This would explain why electrical stimulation of the cut optic nerves of dark-adapted fish, in the light only, led to the usual changes in the retina. Although no determinations were made, it follows that if this hypothesis is true, the converse experiment with light-adapted fish in the dark should result in the assumption of the dark phase on the part of the retinal elements.

Assuming that the balanced action of a system like the one suggested is indeed a reality, it is evident, nevertheless, that questions relative to its adaptive significance are not easily answered. It is certainly difficult to explain the rationale of a situation whereby an animal possesses a mechanism the components of which act antagonistically, thus allowing photomechanical influence to be exerted undisturbed.

Since structure and function go hand in hand, the value of physiological evidence always becomes much enhanced by the coexistence of a correlated structural basis. The possibility of double conduction in one set of nerve fibers is hardly to be considered, hence there arises a pertinent query relative to the anatomical proof for the existence of efferent components of the optic nerve.

Englemann ('85) first postulated 'retino-motor' nerve fibers to explain certain conditions which he asserted occurred in the frog, and further suggested that the anterior arcuate commissure of Hannover in the chiasma, the physiological significance of which had previously been unknown, served as an association tract through which the movements of the retinal elements of the two eyes were interrelated.

⁹ It will be remembered in the experiments on Abramis and Fundulus (p. 233) that after severing the optic nerves the usual responses to light and darkness were maintained. Hence it seems probable (since the retinal elements undergo no changes in excised eyes) that the direct action of darkness as well as light is effective, provided normal circulatory conditions are maintained.

By the use of Golgi methods and by methods of primary and secondary degeneration, centrifugal fibers originating in the vertebrate brain and extending to the retina can be demonstrated. The following quotation from Johnston ('06, p. 265) summarizes the general results gained from studies of this kind:

In fishes in which one eye has long been lost the optic tract of the opposite side degenerates with the exception of these efferent fibers, which persist and are stained by the Weigert method. In mammals, following section of the optic tract there occurs secondary degeneration of cells in the anterior quadrigeminum, and in the dorsal part of the geniculatum laterale and pulvinar. These findings in mammals agree with those in fishes by the Golgi and degeneration methods, where the centrifugal fibers arise from the tectum opticum and geniculatum (Catois, '02). The significance of these fibers is not understood but their presence in all vertebrates seems to show that they have some constant function.

A further anatomical difficulty is presented by the fact that a connection between optic nerve fibers and the pigment cells has never been demonstrated, for even the efferent fibers shown by Cajal ('94) and others have their endings near the internal nuclear layer. Gatten ('07, p. 85) suggested that an actual connection might not be necessary: "Natürlich liesse sich behaupten, die centrifugalen Opticusfasern rufen in der Stäbchenzapfenschicht einen Erregungsvorgang hervor, der sich 'per contiguitatem' dem Pigmentepithel mitteilt." In view of the tentative conclusion reached by me concerning the balanced action of the 'retino-motor' and the inhibitory nerve fibers, it is difficult to state what anatomical conditions would be imposed by such a system. Such matters of detail need not stand in the way of the fact of fundamental importance, which is the existence of demonstrably functional efferent optic nerve fibers.

The significance of these efferent nervous elements in the light of the theory of nerve components may appear to demand no serious consideration, since the majority of neurologists prefer not to homologize the optic 'nerve' with true cranial nerves. Neither the afferent nor the efferent fibers of the optic nerve are comparable to the components of a true peripheral nerve, for both lie within the primary optic apparatus, which is itself a differ-

entiated portion of the brain. Johnston ('06) and Herriek ('15) have assigned the optic nerve proper to the central system of tracts of the somatic afferent division. From the conclusion previously drawn concerning the function of the efferent optic nerve fibers, it is evident that they may, at least with as much propriety, tentatively be called visceral efferent elements.

SUMMARY

When the optic nerve only of *Ameiurus* is severed, the rods, cones and retinal pigment fail to execute their characteristic photomechanical responses. In other words, the movements of the retinal elements depend upon the maintenance of the integrity of this nerve. After hemisection of the nerve, movements of the elements occur only in that region of the retina adjacent to its intact side. It can not only be shown (since essentially normal responses occur in excised eyes and in eyes attached to the body by the optic nerve alone) that a second mechanism exists in association with the muscles innervated by the oculomotor nerve which inhibits these movements when the optic nerve is cut, but also that electrical stimulation of the peripheral stump of the optic nerve can overcome this inhibition.

Hence experimental evidence has been advanced which offers physiological proof for the existence of functional efferent nerve fibers in the optic nerve of *Ameiurus*. Only by the balanced interaction of these elements with a second extrinsic set of nerve fibers (possibly the ciliary nerves), which independently exert an inhibitory effect upon the retinal elements, are normal photomechanical movements of the rods, cones, and retinal pigment accomplished. Although light is ineffectual after section of the optic nerve, temperature produces essentially normal responses in both the pigment and visual cells. It is probable that efferent impulses in the optic nerve fibers do not directly stimulate the retinal elements to motion, but rather such impulses have an indirect action, possibly by counteracting, that is, blocking, the tonic inhibition exerted by the second system of nerve fibers. If these efferent optic nerve fibers fit at all into the scheme of

'nerve components,' they may be designated as visceral efferent elements.

Severance of the optic nerve of certain other fishes (*Abramis* and *Fundulus*) has no inhibitory effect upon the movements of the retinal pigment or of the rod- and cone-cells, and a similar relation has been reported for the frog by other workers. Hence it is impossible to state the extent to which the mechanism discovered in *Ameiurus* may be distributed throughout the vertebrate group, if, indeed, it is not peculiar to *Ameiurus* alone.

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ABBREVIATIONS

<i>ell. bac.</i> , rod ellipsoid	<i>prs. dst. bac.</i> , outer member of rod
<i>ell. con.</i> , cone ellipsoid	<i>prs. dst. con.</i> , outer member of cone
<i>mb. lim. ex.</i> , external limiting membrane	<i>rdx. n. opt.</i> , root of optic nerve
<i>my. bac.</i> , rod myoid	<i>rtn.</i> , retina
<i>my. con.</i> , cone myoid	<i>scl.</i> , sclera
<i>n. opt.</i> , optic nerve	<i>st. bac. con.</i> , layer of rods and cones
<i>pd. cl. pig.</i> , base of pigment cell	<i>st. nl. ex.</i> , external nuclear layer
	<i>st. pig.</i> , pigment layer

PLATE I

EXPLANATION OF FIGURES

The figures of this plate are photomicrographs. Figures 1, 2, 3, and 4 are magnified 160 diameters, figure 5, 46 diameters, figure 6, 19 diameters, and figure 7, 50 diameters.

1 Shows the distribution of the retinal pigment of *Ameiurus* at 5° C. in the light.

2 Shows the distribution of the retinal pigment of *Ameiurus* at 25° C. in the light.

3 Shows the distribution of the retinal pigment of *Ameiurus* at 5° C. in the dark.

4 Shows the distribution of the retinal pigment of *Ameiurus* at 25° C. in the dark.

5 A portion of a section passing through the retina of a previously dark-adapted *Ameiurus*. In the region marked X, close to the optic nerve, a small cut had been made through the eyeball and retina. When the fish was subjected to daylight for 1½ hours, the pigment peripheral to the incision did not expand, as is shown at the right of X in the figure. The pigment in the regions of the retina the optic nerve fibers of which were not affected by the incision expanded essentially in a normal manner, as is shown in the left half of the figure.

6 A section through the entire retina of *Ameiurus*. After the optic nerve of the previously dark-adapted animal had been one-half severed, the animal was exposed to daylight for 2 hours. In the half of the retina at the right of the figure, which was adjacent to the intact portion of the nerve, the pigment migrated to an extreme distal position, leaving an area behind relatively free from pigment. In the left half of the retina, which was adjacent to the cut side of the nerve, the pigment migrated, but not as completely as on the other side. This section passed close to the optic nerve but not through it.

7 A portion of a radial section through the retina of *Ameiurus*. After the optic nerve of a previously light-adapted animal had been two-thirds severed, the fish was subjected to total darkness for 2 hours. At the expiration of this time, the pigment in the half of the retina adjacent to the cut was found to have remained in the expanded position characteristic of light (shown at the right of the optic nerve in the figure), whereas in the half of the retina adjacent to the intact side of the nerve, normal pigment contraction occurred (shown at the left of the optic nerve in the figure).

LESLIE B. AREY

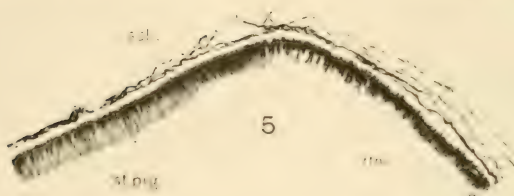


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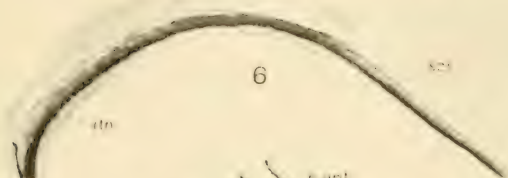
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--- pd. cl. pig.
st. pig.
--- mb. lim. ex.



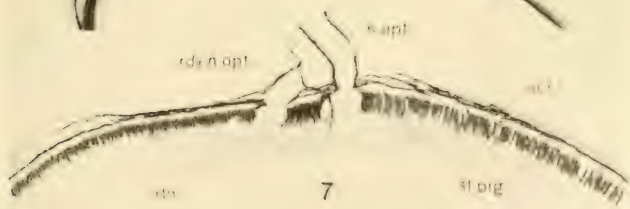
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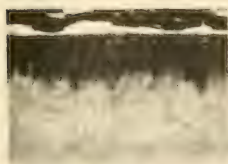
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6



7



3

--- scl.
--- pd. cl. pig.
st. pig.
st. bac. con.
--- mb. lim. ex. ---



4

PLATE 2

EXPLANATION OF FIGURES

All drawings were made at a magnification of 1400 diameters, a Leitz $\frac{1}{12}$ homogeneous immersion objective being used; in the reproduction figures 8, 9, and 12 are reduced to a magnification of 1150 diameters, and figures 10 and 11 to 950 diameters.

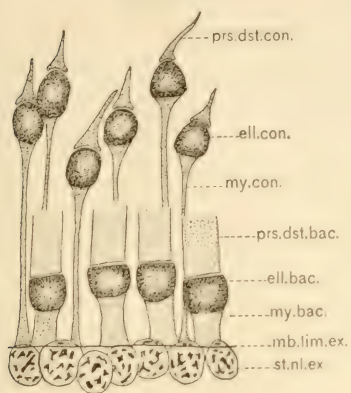
8 Shows the positions assumed by the visual cells at 3° C. in the dark.

9 Shows the position assumed by the visual cells of *Ameiurus* at 27° C. in the dark.

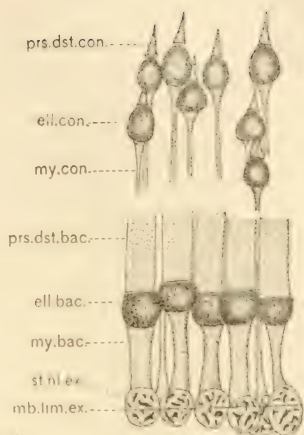
10 Shows the characteristic position of the visual cells of *Ameiurus* at room temperature in the dark.

11 Shows the characteristic position of the visual cells of *Ameiurus* at room temperature in the light.

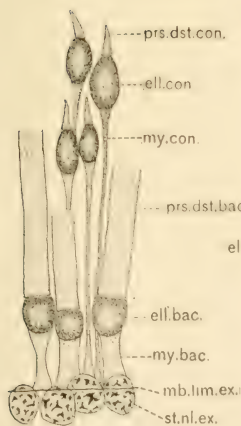
12 From the retina of a dark-adapted *Ameiurus* the optic nerve of which had been cut and the animal subjected to light. The rods remain shortened, as in darkness, although in this case the cones have shortened to a great extent, as is the characteristic response in light.



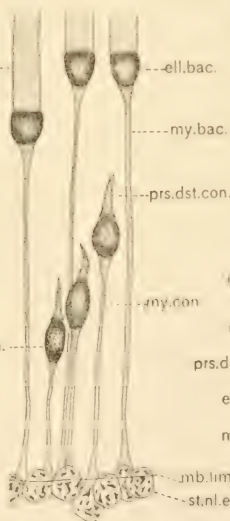
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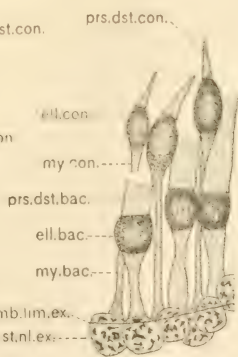
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10



11



12

L. B. A. Del.

CORRELATED ANATOMICAL AND PHYSIOLOGICAL STUDIES OF THE GROWTH OF THE NERVOUS SYSTEM OF AMPHIBIA

II. THE AFFERENT SYSTEM OF THE HEAD OF AMBLYSTOMA

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SEVENTY-NINE FIGURES

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The first paper of this series, "The Afferent System of the Trunk of *Amblystoma*," was published in Volume 24, number 2 (1914), of this Journal. It will be referred to in this communication as Paper I. In it (p. 163) were described the four physiological stages of development upon which this paper, also, is based. The next paper of the series will deal with the neurones of the second and higher orders in the reflex arcs of the brain and spinal cord.

I. THE ANATOMICAL PART

This part of the paper involves a rather detailed description of the sense organs and sensory nerves of the head. This description, however, is not exhaustive, and may require supplementing as the study progresses. Readers who desire an even briefer treatment of this phase of the subject may turn to the summary of this part of the paper (p. 276) and to the figures, in connection with which salient anatomical features are mentioned.

1. *The trigeminal nerve*

A. THE NON-MOTILE STAGE

In embryos of the non-motile stage the ophthalmic ganglion and the Gasserian ganglion are situated widely apart and come into relation with each other only through their roots as the latter approach the brain. The double nature of the trigeminal nerve, therefore, is very obvious. As shown in figure 1, the ophthalmic ganglion already extends forward over the eye and the Gasserian ganglion extends ventrad behind the eye.

The ophthalmic ganglion is directly continuous with the skin near its rostral end (figs. 1 and 7 to 10, *Ad., G.oph.*). Favorable plane of section reveals two rather distinct portions of this ganglion (figs. 10, 11, 12, *G.oph.a,b*), and, at least in some specimens, each part has an area of adhesion with the skin. Two such areas are indicated in figure 1 (*Ad.G.oph.*).

No peripheral distribution of fibers from the ophthalmic ganglion to the skin can be made out at this stage. The neuroblasts of the ganglion are bipolar and have perceptible axone and

dendritic processes. The structure of one of these cells, taken from the most distal portion of the ganglion, is shown in figure 18. The proximal portion of the cell is not clearly seen in the section figured, but the dendritic pole extends well out into mesenchyme which surrounds the ganglion. In adjacent sections it can be followed as a delicate fibrillated strand of protoplasm about three times the extent of the process as shown in the figure. The course of this fiber, however, is not directly towards the skin and it becomes lost to view among the cells of the mesenchyme. I have been unable to find in this stage of the embryos any highly branched endings of naked fibers like the terminals of the fibers of the giant ganglion cells of the trunk (Paper I, figs. 17, 23 to 25). It may be that exceedingly fine filaments of protoplasm from the ganglion cells reach the skin here and there; but if they do, they escape the most exhaustive search through favorable preparations. The only perceptible connection, therefore, of the ophthalmic ganglion with the skin in the non-motile stage is through the areas of adhesion, or continuity with the ectoderm as described above.

The attenuation of the ophthalmic ganglion caudad into a slender root is illustrated in figures 1, 7 and 8 (*G.oph.*, *R.oph.*). Caudally of the region indicated in figure 8 the root becomes more slender still, so that it is very difficult to identify among the mesenchyme cells. In frontal sections, however, it is easy to trace the root to the brain in two closely associated divisions which correspond to the two portions of the ganglion (figs. 11 and 12). As it nears the brain the root bends dorsad slightly and approaches the brain in close association with the root of the Gasserian ganglion but at a slightly more ventral level than the latter.

As illustrated in figure 1, the Gasserian ganglion lies well out from the brain with its long axis directed latero-ventrad. There is at this time no perceptible development of fibers to form a nerve trunk beyond the distal limits of this ganglion (fig. 1). As in the case of the ophthalmic ganglion, there is here also an area of adhesion between the ganglion and the skin. This contact is at the distal end of the ganglion (fig. 9, *Ad.G.G.*). It is

not as extensive or as intimate as the adhesion between the ophthalmic ganglion and the skin. In a transverse section of the embryo this adhesion is seen in figure 9 to be ventral of the primordium of the preauditory lateral line organs.

In the first section ($10\ \mu$) ventrally of that of figure 12 the root of the Gasserian ganglion enters the brain, while in the next following section ($20\ \mu$) the root of the ophthalmic ganglion enters. The axones of neither of these roots have grown beyond 10 or $20\ \mu$ from the immediate zone of entrance. Throughout their extent, however, they form a dense fibrillar mass immediately beneath the external limiting membrane of the brain. There is no clear evidence of bifurcation of the root fibers at this stage. The axones which compose the root of the Gasserian ganglion are illustrated in figure 19. The cells figured here occur a considerable distance out from the brain and the longer processes are the axones. The axones of the two cells of the figure come into such close relation with each other that they might be interpreted as fusing together into a single root fiber, but they probably do not anastomose. The perikarya of these neurones or neuroblasts, like those of all other ganglia of this age, are filled with yolk spherules.

As the roots of the ophthalmic and Gasserian ganglia approach the brain a small strand of fibers enters them from a more dorsal position in the brain. The neurones from which these fibers arise lie just inside the external limiting membrane and, mostly at least, in a more dorsal position than the root entrance. They have large spherical nuclei and the other morphological characteristics of the Rohon-Beard cells of the spinal cord. In figure 20 (*DC*) one of the these cells is shown in a transverse section of the medulla oblongata, and $15\ \mu$ caudad of this appears another such cell, which is reproduced in figure 21. The former neurone sends its process to the immediate region of the entrance of the trigeminal root while the latter (fig. 21) sends its process through the external limiting membrane into the root of the nerve. There are in this section two or three other neurones of this type. At this stage the cell boundaries are rather indistinct, but the fibrils which compose the emerging fibers of figure 20 con-

verge towards the limiting membrane from among the yolk globules that surround the nucleus. These yolk globules are intracellular and certainly belong to the cell of this nucleus, so that this strand of fibrils interspersed with yolk globules must be a process of the cell.

That these neurones with processes running out into the trigeminal nerve are not motor is clearly shown by reference to figure 20, in which the motor nucleus of the nerve is easily established in a much more ventral position (*Nuc.Vis.m.*). The cells in question, therefore, must be giant ganglion cells, representing an extension of the Rohon-Beard cells of the spinal cord into the brain. As to the peripheral distribution of the fibers from these cells, nothing can at present be said excepting that they appear to enter both divisions of the trigeminal nerve.

In this and in some of the later stages there appears just outside the brain and about the roots of the trigeminal nerve particularly, but in a less degree about the roots of the other nerves, a mass of cells which is quite distinct from the ganglia. It is made up largely of indifferent cells, but cells resembling neuroblasts occasionally occur in it. This structure I have called the root mass. It is indicated (*R.M.*) in figures 1, 2, 12, 15, 20 to 22, 34 to 37, 47 to 49, 69. There are no apparent grounds for assigning to it any physiological significance, and I have made no effort to determine its origin, history or morphological significance.

B. THE EARLY FLEXURE STAGE

By reference to figures 1 and 2 it will be seen that the ophthalmic ganglion has drawn somewhat nearer the brain. The position of the ganglion with reference to the eye is further illustrated in figure 13 (*G.oph.*), and the attenuated nature of its root at this stage is shown in figure 14 (*R.oph.*). The ganglion has now broken away from its adhesion with the skin, but thickened regions of the ectoderm indicate the areas of earlier attachment (*Ec.Th.*). One of these ectodermal thickenings is shown in figure 13. The two parts of the ophthalmic ganglion are still perceptible.

The ophthalmic trunk has grown considerably rostrad from the ganglion (fig. 2, *o.p.V*), and follows a course in conformity with the contour of the eye. It forms two divisions as it leaves the ganglion, these representing, apparently, the two parts of the ganglion that have been noted above. The larger of these two nerves follows closely the contour of the eye to the immediate region of the olfactory epithelium. Along its course several filaments arise which penetrate the surrounding mesenchyme, with the cells of which they become so intimately associated that they very rarely can be traced continuously to the skin. From among the mesenchyme cells, however, naked filaments emerge beneath the skin and attach themselves to it. Such filaments are in all probability the terminals of the n. ophthalmicus profundus. They have the appearance of terminals of the Rohon-Beard cells in the trunk, as described in Paper I. It is possible that they are processes from sensory neurones in the medulla oblongata such as we have here under consideration. The smaller of the two divisions of the N. ophthalmicus profundus passes more directly forward (morphologically dorsad) and can be followed only indefinitely in its distribution (fig. 2, *t*).

The Gasserian ganglion still projects ventrad caudally of the eye (fig. 2, *G.G.*), and dorsally of the primordium of the muscles of mastication (fig. 15, *G.G.*). An infraorbital trunk now arises from the extremity of the ganglion and extends at least two-thirds of the distance across the lateral surface of this primordium (fig. 2, *Mdb.*). An occasional fiber arises from this nerve and passes towards the skin, but no definite cutaneous distribution can be made out for the nerve as a whole.

The root fibers of the trigeminal nerve, upon entering the brain, form an ascending and descending trigeminal tract (fig. 22, *Tr. asc.V, Tr. des.V*). These apparently arise by a bifurcation of the root fibers, although one can not be positive of this upon the basis of the observations of individual fibers since the fibers are very compact in their grouping. Figure 22 shows favorably the extension of the trigeminal root fibers into the brain. The plane of section may be interpreted from figure 16, which was made from the same section. It will be seen to pass longitudinally

through the medulla oblongata and to be inclined ventro-lateral from the middle line on the side viewed in figure 22. This plane of section reveals characteristic ventricular pits opposite the entrance of the nerves of the trigeminal and facial groups. The extent of the ascending trigeminal tract is probably fully shown in figure 22 (*Tr.asc.V.*). It extends only a short distance rostrad immediately beneath the external limiting membrane. The descending trigeminal tract (*Tr.des.V.*) is considerably longer and of a more complicated structure. As illustrated in figure 22, this tract runs from the trigeminal root to the level of the middle of the auditory vesicle immediately next to the external limiting membrane. The development of the tract is even slightly exaggerated in the figure. The trigeminal root fibers themselves appear to extend only about to the level indicated in the figure by the reference line *Tr.Des.V.* Beyond this the tract appears to be made up of short processes of neuroblasts along its course, corresponding, presumably, to neurones of an incipient substantia gelatinosa. The fibrillar element of this tract can be recognized only in favorable longitudinal sections. Its full description belongs to a later paper.

A gaint ganglion cell component of the trigeminal nerve exists also in this stage, but nothing noteworthy appears concerning either its central or peripheral relations.

C. THE COILED-REACTION STAGE

The ophthalmic ganglion has receded along the root till its proximal portion has become massed against the brain. Its relation to the eye, also, is more intimate than in the earlier stages (figs. 3 and 76, *G.oph.*). The n. ophthalmicus profundus is perceptibly more developed than in the early-flexure stage and the distribution of its fibers to the skin is more obvious. In addition to the two main divisions branching out in front of the eye there are now fibers that go directly from the ganglion to the overlying skin. The root fibers can be traced into the brain and differentiated from the root fibers of the Gasserian ganglion, the latter entering in a slightly more dorsal position.

The Gasserian ganglion, also, has migrated towards the brain. The infraorbital trunk can be traced approximately to the ventral margin of the primordium of the muscles of mastication (fig. 3, *Mdb.*) It now passes through a distinct groove in this primordium. As it leaves this groove it gives off the ramus maxillaris (*Mr.V*) which passes only a short distance forward under the eye. No fibers from the r. mandibularis can be traced into the balancer at this stage. The fibers to the skin in the post-optic region are still less obvious than they are in front of the eye, but favorable preparations show that they are present.

Figure 33 illustrates the trigeminal root as it enters the brain. The plane of section of this figure may be judged by figure 17, in which the area of figure 33 is blocked out and marked *a*. This plane of section will be recognized as inclined cephalo-ventrad in a longitudinal direction. The small ascending division of the root (*Tr.Asc.V*) can be recognized in the figure and the appearance of the ascending tract in transverse section is shown in figure 34, which is $56\ \mu$ rostrally of the entrance of the root. Here the ascending fibers are found scattered through a considerable area dorso-ventrally. The descending division of the root (fig. 33, *Tr.Des.B*) is much more massive than the ascending, and extends caudad to about the level of the facial root, as shown in figure 5, sketched in solid black. The tract which is labeled *Tr.Des.V.* in figures 38 to 49 is apparently composed at these various levels of processes of central neurones. Its fibers are scattered through a considerable area dorso-ventrally and as such a diffuse system may be followed on caudad into the spinal cord.

The giant ganglion cell component of the nerve is more obvious in this than in earlier stages. In figure 33 one of these ganglion cells (*DC*) appears in the angle of bifurcation of the root of the ganglionic component. The cell boundaries are here clearly defined. The plane of section, already mentioned as illustrated in figure 17, is such that the rostral (right) end of the figure is considerable more ventral than the caudal (left) end, and motor cells appear in the more ventral portion of the figure (*Nuc.vis.m.*). In figures 35, 36 and 37, three successive sections in the trans-

verse plane, through the entrance of the trigeminal nerve, the same type of cell appears. In figures 36 and 37 the same cell appears (*DC*). Its typical, large nucleus has two nucleoli, one in each section. In both sections the peripheral process, which projects into the root, can be distinctly recognized. In figure 36 a process runs ventrad towards the motor centers, and in figure 37 there appears the basal portion of a process which is directed dorsad. This structure conforms to the Rohon-Beard cells which, in early stages, have one peripheral process and two central. Figure 35 shows the presence of several other cells of this type in the immediate vicinity of the root. The distinctly more ventral position of the motor nucleus (fig. 36. *Nuc.vis.m.*) precludes any possibility of confusing motor with sensory cells in this region. In figure 34 there is represented one of these cells which is situated still farther dorsad, and $56\ \mu$ cephalad of the entrance of the root of the nerve. The long process running ventrad from this cell indicates that it has become unipolar, after the manner of the Rohon-Beard cells in the spinal cord.

D. THE EARLY SWIMMING STAGE

In embryos of this stage the ophthalmic ganglion has pressed still farther back upon the root against the brain. It now has a wide contact with the Gasserian ganglion (fig. 4, *G.oph.*). Cephalad it extends nearly to the rostral border of the eye (fig. 78). From the caudal portion of the ganglion there now arises a small nerve which goes dorsad to the skin. In some specimens this seems to be represented by a single fiber. It appears as such in silver impregnations. This is probably my branch 'x' of the advanced larva and adult ('02). Near the rostral border of the eye (fig. 4, *L.o.p.V*), a branch, consisting of only a few fibers, passes laterad around the anterior surface of the eye to a position lateral and caudal of the external nares. This is presumably my lateral terminal branch of the adult ('02, fig. 2, *L.o.p.V*). The remainder of the nerve passes along the mesial aspect of the olfactory epithelium to the skin of that region. This must be the mesial terminal branch of the adult. A con-

siderable number of fibers of this nerve attach themselves in a cluster to the supraorbital lateral line primordium, to which the course of the nerve has a definite relation. These branches from the ophthalmic nerve have all been identified in silver impregnations.

The root fibers of the ophthalmic ganglion can be traced to the brain and can be seen to enter the brain a little ventrad and cephalad of the entrance of the root of the Gasserian ganglion (figs. 51 to 53, *R.oph*). The intimate relation between the proximal portions of these two ganglia is shown in these figures.

The Gasserian ganglion, like the ophthalmic, has become more consolidated about the proximal portion of its root than in any of the earlier stages. The infraorbital trunk has become still more deeply embedded in the groove through the primordium of the jaw muscles, across the entire lateral surface of which it now descends to the vicinity of the balancer. To the balancer it sends a nerve (fig. 4, *c*), which can be traced in both stained preparations and silver impregnations, practically to the tip of the organ where it has cutaneous distribution. About where the main nerve leaves the groove it gives off the ramus maxillaris (fig. 4, *Max.V*) which now passes a considerable distance forward under the eye. There is some evidence that this nerve arises from cells that lie in the angle between the main projections of the ophthalmic and Gasserian ganglia. Soon after giving off the nerve to the balancer the ramus mandibularis divides into two branches which turn cephalad and mesad ventrally of the muscle primordia. These can be traced into close proximity with the oral plate. In silver impregnations of embryos of this age neurofibrils may be seen penetrating the skin along the course of this nerve. The cutaneous distribution of the nerve, however, is still less obvious than is that of the ophthalmic division, excepting in the balancer, which is a highly innervated organ at this stage.

The root fibers of the Gasserian ganglion are shown in figures 51 to 54 as they enter the brain to form the more dorsal portion of the ascending and descending trigeminal tracts. The extent of these tracts is shown in figure 6 (*Tr. asc.V*, *Tr. des.V*). Silver

impregnations, which differentiate these root fibers clearly from the smaller fibrils of the region, show them running cephalad almost through the metencephalon and caudad beyond the level of the auditory vesicle. Along this course fibers of smaller caliber group themselves along the deeper aspect of the root fibers. The tract is, therefore, a composite of sensory neurones of the first and second orders. Just how far the root fibers extend can not be exactly determined, but they appear to reach the level of the general cutaneous root of the vagus, and some of them may even reach the spinal cord. The descending trigeminal tract as a whole is continuous with the sensory tract of the spinal cord (figs. 6, 66, *DT*, *Tr.Des.V*).

In embryos of this age silver impregnations have been made which show the processes of giant ganglion cells extending continuously from the cell body into the root of the trigeminal nerve. Cells of this description are situated dorsally of the root and in the root entrance zone in figures 51 to 54 (*DC*). In figure 50 there is a representative group of these cells which are situated 35μ caudad of the root of the nerve. The ventrally directed processes of the cells are here shown in their relation to the deeper face of the descending trigeminal tract.

In figures 51 to 54 there is illustrated, also, in a general way the degree of differentiation of neuroblasts and neurones in the marginal zone of the substantia grisea, and the elevated regions in the ventricular floor in some parts as compared with the relative flatness in others. The study of the interrelations of such centers of differentiation and ventricular eminences belongs to a later paper. It may be added here, however, that fine terminals from the trigeminal root fibers ramify among the neurones which are grouped along the trigeminal tract.

2. *The facial and auditory nerves*

In all four stages of development here under consideration the various elements of this ganglionic complex can be recognized: the lateral line and visceral components of the facial and the auditory.

A. THE NON-MOTILE STAGE

1. *The lateral line component.* The position and extent of development of the primordia of the various lateral lines of the head are shown in Paper I, figure 56 (*Pr.LL.*). The primordium of the preauditory lines consists of a single boot-shaped patch of thickened epithelium: the toe of the 'boot' extending forward and a little dorsad as the beginning of the supraorbital line and the heel projecting ventrad as the beginning of the infra-orbital line. From the caudal end of this structure a narrow strip of thickened epithelium connects it with the auditory vesicle, which still has a broad attachment with the skin (fig. 73, *Ad., Aud.V*). This slender connective between the auditory vesicle and the lateral line primordium has the structure of the thickening of ectoderm which persists for a time after the ophthalmic ganglion detaches itself from the skin. It may represent the area of origin of a dorso-lateral placode, although I have no conclusive evidence of this.

The facial lateral line ganglia of this stage are rather loose masses of cells to which only indefinite borders can be established (fig. 1). The mass of cells which represents the hyomandibular division of the nerve extends a considerable distance caudad under the auditory vesicle, with which it seems to be fused in a small area near the auditory ganglion. From this region it projects laterad and rostrad as a loose collection of cells which has a very indefinite relation to the skin. The other lateral line facial ganglion, which later gives rise to the r. ophthalmicus superficialis VII and r. buccalis VII has a more definite outline and is somewhat more highly differentiated. It holds a more dorsal position and extends rostrad to the lateral line primordium, with which it is in direct contact. A considerable number of fibers from the ganglion attach themselves to the primordium, upon which they can be traced for only a very short distance.

The lateral line ganglia have root fibers which reach to the surface of the brain at about the level of the rostral border of the

auditory vesicle, but within the brain these fibers have no perceptible extension.

2. *The visceral sensory component of the facial nerve.* The ganglion of this component, also consists of rather scattered cells which occupy a position ventrally of the lateral line ganglia. It projects laterad and ventrad to the ectodermal thickening which is associated with the spiracular pouch (Paper I, fig. 56 *Ec.Th.*). With the cells of this thickening the ganglion is practically continuous. There are no perceptible nerve fibers in the peripheral portion of the ganglion or arising from it to form a nerve. Centrally there appear to be a few root fibers which reach the brain, but it has so far been impossible to differentiate them certainly from the other root fibers of the complex or to establish that they are constantly developed at this time.

3. *The auditory nerve and vesicle.* The auditory vesicle of this stage has a broad attachment to the skin (fig. 73, *Ad., Aud.V*). This contact is with the lateral aspect of the vesicle. The endolymphatic appendage (*End.*) is very imperfectly differentiated from the rest of the vesicle, but it can be readily recognized. The epithelium of the mesial, ventral and ventro-lateral regions of the vesicle is still very thick, and mitosis is particularly abundant in the more ventral portion. The ventro-mesial portion of the vesicle is continuous with the auditory ganglion (fig. 73, *G.VIII*). As the ganglion projects rostrad it become free from the vesicle and a fiber or two from its proximal end reach the brain at a point more ventral and caudal than the root connection of the lateral line ganglia with the brain.

B. THE EARLY FLEXURE STAGE

All the ganglia of this complex have acquired more definite contours and greater compactness of structure. The interrelations of the ganglia can be most favorably studied in frontal sections; the root connections with the brain, in transverse sections.

1. *The lateral line component.* The two, distinct lateral line ganglia I distinguish as a and b (fig. 2, *G.L.L.VII,a* and *b*). The

degree of separation of these two ganglia is somewhat exaggerated in figure 2. Ganglion a is distinctly farther advanced in development than ganglion b. In horizontal sections it exhibits a line of cleavage between two portions, and each portion gives rise to a distinct fiber bundle. These two bundles emerge from the distal end of the ganglion and obviously represent the r. ophthalmicus superficialis VII and the r. buccalis VII. The latter is directed the more ventrad. There are now sheath cells upon the fiber bundle from the ganglion to the lateral line primordium, and closely attached to the latter the naked fibers extend for some distance in two strands, representing the two nerves as indicated. The primordia of the lateral lines have shifted farther rostrad relative to the position of the auditory vesicle, from which they have become completely detached (Paper I, fig. 57, *Pr.LL*). Their position, also, is somewhat more dorsal. Their ventral projection is distinctly larger and shows a tendency to form into two divisions.

Ganglion b, of the hyomandibular division, is still very imperfectly differentiated in its proximal portion from the auditory ganglion. Distally it is seen in frontal sections along the caudal and lateral border of the geniculate ganglion (fig. 2, *G.gen.*), the point of it extending ventrad behind the attachment of the entoderm of the spiracular pouch to the ectoderm. From this point of the ganglion a few fibers extend on ventrad and into the primordium of the hyoid arch. These fibers can be recognized only as they run in the plane of section. The fact that these fibers have no perceptible connection with the skin and that the primordium of the mandibular lateral line has not yet appeared leads me to interpret them as probably motor.

The root fibers of the lateral line ganglia still enter the brain at a slightly more rostral level than the auditory vesicle, and in two divisions (figs. 23 to 28, *R.L.L.VII,a* and *b*). The dorsal division seems to arise from ganglion a; the ventral division, from ganglion b. In figure 23 a few ascending fibers of each division can be seen (*L.L.VII,Asc.,a,b*). In figure 28 the corresponding descending divisions of these root fibers are represented (*L.L.VII,Des., a* and *b*). Presumably the fibers bifurcate

upon entering the brain at this stage, although this has not been actually demonstrated in individual fibers.

2. *The visceral sensory component.* The geniculate ganglion in its proximal portion holds a relatively ventral position in the ganglionic complex. Distally it reaches ventrad and rostrad to the region of contact between the ectoderm and entoderm of the spiracular pouch, where it becomes continuous with a cluster of ectodermal cells which has pushed in over the entoderm. This cluster of cells is, presumably, the epibranchial placode of this nerve. No fibers can be seen issuing from the geniculate ganglion at this stage. The root fibers of the geniculate ganglion are shown approaching the brain in figure 25 (*R.VII.vis.*) at a distinctly more ventral level than the lateral line root. They turn caudad immediately within the external limiting membrane and form an incipient fasciculus solitarius (figs. 26, 27). Their visible course is limited to the sections figured in this series.

3. *The auditory organ and nerve.* The auditory ganglion has become completely detached from the vesicle. In figure 75, a single cell (*VIII*) represents the distal end of the ganglion. Towards the brain from this position the ganglion can be followed distinctly (fig. 2, *G.VIII*). The auditory vesicle has detached itself entirely from the skin and has made very perceptible progress in differentiation since the non-motile stage, as may be seen by comparing figures 73 and 75. Mitotic figures are still very abundant in the inner portion of the epithelium. The root fibers of the nerve are seen in figure 27 (*R.VIII*) entering the brain on the ventral aspect of the root of the geniculate ganglion. In figure 28, taken from the next section caudad of 27 ($10\ \mu$) no descending auditory root fibers can be definitely recognized.

C. THE COILED-REACTION STAGE

As in the case of the trigeminal ganglia, the ganglia of the facial complex have become more consolidated towards the brain and around the antero-ventral aspect of the auditory vesicle.

1. *The lateral line component.* The lateral line primordia have become greatly extended since the early flexure stage (Paper I,

fig. 58). In addition to the primordia shown in the latter figure there is now a primordium which runs mesad from the base of the balancer on the ventral surface of the head. In this epithelial thickening there is a lowly differentiated lateral line organ at the mesial end and possibly another at the base of the balancer. This primordium is not connected with the other preauditory primordia, unless it is by a very slight ectodermal thickening of an indifferent nature.

The lateral line ganglia *a* and *b* are in close contact with each other proximally but project distally into distinct ganglia (fig. 3, *G.L.L.VII,a,b*). Ganglion *a* still reaches almost to the skin. In its distal portion it still has two parts, from each of which arises a nerve. These nerves follow the primordia closely attached to the skin for a considerable distance. The finer divisions have no sheath cells and their exact length is therefore difficult to determine. Ganglion *b* projects ventrad behind the spiracular pouch and from it nerve fibers pass to the skin at or near the lateral line primordium which has been described as occurring in this stage at the base of the balancer.

The roots of the lateral line ganglia enter the brain as illustrated in figures 38 to 41, figure 38 being the most rostral of the series. Here the root fibers of ganglion *b* (*R.L.L.VII,b*) are entering the brain and collecting into a bundle immediately beneath the external limiting membrane. In figures 39, 40, and 41, the next successive sections caudad, the root fibers of ganglion *a* are entering in a more dorsal position (*R.L.L.VII,a*). Within the brain these fibers appear to bifurcate. At least they form short ascending and descending tracts as shown in figure 5 (*L.L.Asc.,Des.,a* and *b*). Figure 38 shows the ascending tracts, and figure 41, the descending tracts in cross section, under high magnification.

2. *The visceral sensory component.* The proximal portion of the geniculate ganglion has not changed its position noticeably (fig. 3, *G. gen.*). Distally it projects to the spiracular pouch, where its appearance has materially changed. Along the caudal aspect of the entodermal wall of the pouch a fiber or two may be traced along the bases of the entodermal cells. Their course, however,

is very short, for they can not be traced into the hyomandibular trunk. They take a course in conformity with this trunk, and probably represent an incipient r. alveolaris. This part of the ganglion apparently corresponds to the geniculate ganglion which has been described in the earlier stages. In addition to this there is now a very massive connection of the ganglion with the ectoderm running forward over the pouch and at the same time in close relation with the entoderm. The structure here gives one the impression that the ectoderm is contributing large numbers of cells to the ganglion. This is probably the epibranchial placode, although my specimens have not been selected at short intervals and are not adequate for the deciding of this question. No fibers emerge from this portion of the ganglion.

The root of the geniculate ganglion is shown entering the brain in figure 39 (*R.VII,vis.*). Within the brain they turn caudad and form a distinct fasciculus solitarius (figs., 39 to 41, *Fas.Sol.*), which lies against the limiting membrane and wedged in between the lateral line tracts dorsally and the auditory tract ventrally. This tract can now be followed to the level of the glossopharyngeal root, from which it receives an increment, and then on to the level of the root of the vagus nerve (fig. 5).

3. *The auditory organ and nerve.* The wall of the auditory vesicle has become greatly reduced in thickness and its lumen much enlarged since the early flexure stage (fig. 77). In the inner portion of the ventral wall mitosis is still going on rapidly. The auditory ganglion is closely applied to the vesicle ventromesially, while the endolymphatic appendage is closely pressed against the brain. This contact is obviously a secondary thing, brought about by the enlargement of the vesicle (compare figs. 73, 75, 77, 79). The auditory nerve can be traced to the caudal extremity of the vesicle, closely applied to the vesicle in about the same relative position as it appears in figure 77. The root fibers (*R.VIII*) enter the brain as shown in figures 40 and 41 ventrally of the root fibers of the geniculate ganglion. Their course caudad on the ventral aspect of the fasciculus solitarius is indicated in figure 5 (*VIII,des.*).

D. THE EARLY SWIMMING STAGE

The facial and auditory ganglia have in this stage become still more consolidated about the auditory vesicle and the roots enter the brain farther caudad relative to the vesicle.

1. *The lateral line component.* The preauditory lateral line primordia have advanced greatly in distribution and differentiation (Paper I, fig. 59). The supraorbital primordium can now be traced far down in front of the eye and the olfactory organ and spreads out at the end, in anticipation apparently of the line of organs that develops later around the rim of the snout. The suborbital primordium extends beneath the eye into close relation with the olfactory organ, while the proximal portion of this primordium is now continuous with the primordium of the hyomandibular region. The latter primordium now passes ventrad behind the balancer, then mesad, where it appears on the ventral surface behind the oral plate in two distinct ridges, in which the characteristic organ structure is well pronounced.

Silver impregnations of the lateral line fibers now show them extending practically the full length of the primordia, and following rigidly the course of the primordia as in the earlier stages. The nerve trunks are everywhere closely adhering to the epithelium and here and there fine fibrils can be followed in among the cells.

The root fibers of the lateral line ganglia impregnate much more deeply with the silver methods than do the peripheral fibers. They enter the brain as illustrated in figures 55 to 60, in two roots, *R.L.L.VII, a* and *b*). From a study of silver impregnations of these fibers it is impossible to be absolutely sure that all fibers from ganglion *a* enter root *a*, or that root *b* is exclusively made up of fibers from ganglion *b*. But if there is an intermingling of fibers from the different ganglia in the individual roots this must be very slight. The roots, upon entering the brain, form ascending and descending tracts, as shown in figure 6. The descending tracts reach the level of the middle of the auditory vesicle, and the ascending tracts are of about the same length or a little longer. The relation of the descending tracts

of the facial to the ascending tracts of the postauditory lateral line roots is shown in figure 61, where the tract VII *a* is most dorsal of the series. Between this and tract VII *b* is the dorsal division of the postauditory roots. Most ventral in the set is the ventral division of the latter. Good silver impregnations in longitudinal section make it appear that these tracts may all be considerably longer than figure 6 represents them.

2. *The visceral sensory component.* The geniculate ganglion is now in contact with the ectoderm by only a slender column of cells which rests also upon the entoderm. This ectodermal contact of the ganglion lies about midway between two other projections of the ganglion: the one contributing fibers to the r. hyomandibularis as the latter passes ventrad in very close relation with the caudal wall of the spiracular pouch (fig. 4, *c*); the other, directed rostrad on the dorsal surface of the entoderm, giving off a well defined r. palatinus (fig. 4, *d*). This nerve passes mesad and a little rostrad over the pharyngeal cavity almost to the middle line and forward to within about 50 μ of the rostral end of the foregut.

The root fibers of the geniculate ganglion enter the brain ventrally of the lateral line roots, as seen in figure 56 (*R.VII, Vis.*) and then turn caudad as the fasciculus solitarius (fig. 6, *Fas.Sol.*). The tract still lies immediately against the limiting membrane. It is distinctly larger than in the coiled-reaction stage.

3. *The auditory organ and nerve.* The endolymphatic appendage is more sharply differentiated from the body of the auditory vesicle than it was in the earlier stage, and is still closely pressed against the brain (fig. 79). The auditory ganglion and nerve extend to the caudal border of the vesicle in the position shown in figure 79. In silver impregnations the fibers of the nerve now appear as exceedingly fine filaments which become closely merged with the basal ends of the epithelial cells or with a basement membrane which is also slightly impregnated. Nerve ending upon the epithelial cells of the vesicle, however, have not been observed.

The root fibers of the auditory ganglion, entering the brain as shown in figures 57 to 60, form a descending tract which extends

along the ventral aspect of the fasciculus solitarius to the level of about the middle of the auditory vesicle (fig. 6).

3. *The glossopharyngeal and vagus nerves*

This complex includes lateral line, general cutaneous and visceral components.

A. THE NON-MOTILE STAGE

1. *The lateral line component.* There are two postauditory lateral line primordia (Paper I, fig. 56, *Po.LL.1* and 2). The one of these is closely associated with the ectodermal thickening over the first branchial pouch; the other lies along dorsally of the thickening of the ectoderm in the vicinity of the more caudal pouches. From the primordium over the first branchial pouch a slender strand of cells reaches to the brain (fig. 1, *G.L.L.IX*, *R.L.L.IX,X*). These cells are laden with yolk spherules and are mostly of an indifferent nature, although some of them have the appearance of neuroblasts. This is the lateral line ganglion of the glossopharyngeus. While the root reaches the brain, nothing can be determined concerning its fibers within the brain.

The main lateral line ganglion of the vagus is a spindle-form body, situated opposite the first and second myotomes. It has a broad attachment with the lateral line primordium of that region. Its root is so slightly developed that it can be recognized only as it lies in the plane of section. A very few root fibers reach the brain in connection with the lateral line root of the ninth nerve (fig. 1, *G.L.L.X*).

2. *The general cutaneous component.* On the lateral aspect of the lateral line ganglion of the vagus is a loose aggregation of cells which extends caudad into very close relation if not into contact with an ectodermal thickening which is practically continuous with the lateral line primordium associated with the vagus nerve. This cluster of cells, representing the jugular ganglion of the vagus, is slightly more condensed in its most rostral portion, which bends mesad ventrally of the lateral line

ganglion. This incipient root, however, does not reach the brain (fig. 1, *G. Jug.*).

3. *The visceral sensory component.* The visceral ganglion of the glossopharyngeus fuses with the ectodermal thickening which rests upon the entoderm of the first branchial pouch (Paper I, fig. 56, *Ec.Th.*). This structure is obviously the epibranchial placode of the glossopharyngeus. From it a strand of ganglion cells follows along the lateral line root to the brain. No definite relation of root fibers, however, can be made out (fig. 1, *G. Vis.IX*).

The visceral ganglion of the vagus nerve is in a still more embryonic condition. It consists for the most part of a loose collection of cells situated on the ventral side of the lateral line and general cutaneous ganglia. The most highly differentiated part of it reaches out to the ectodermal thickening over the second branchial pouch, with which it fuses. Farther caudad there is loose connection of the ganglion with the ectodermal thickening over the third and fourth visceral pouches. The ganglion at this time has no root connection with the brain (fig. 1, *G. Vis.X*).

B. THE EARLY FLEXURE STAGE

1. *The lateral line component.* The lateral line primordium, which is situated over the first branchial pouch, has become elongated dorso-ventrally, and two other primordia appear, the one situated over the second branchial pouch opposite the first myotome and the other opposite the second to fifth myotomes (Paper I, fig. 57).

The lateral line ganglion on the ninth nerve has now become spindle form, but it still reaches to the primordium (fig. 2, *G.L.L.IX*). Its root fibers reach the brain in close contact with the root fibers from the lateral line ganglion on the vagus. The latter ganglion now sends out a projection of cells which reaches to the primordium which is situated over the second branchial pouch, and extends caudad to the primordium of the trunk (fig. 2).

The roots of these postauditory lateral line ganglia approach the brain together (figs. 29 and 30). In figure 30 this root

appears in two parts, a dorsal, from the ganglion on the vagus, and a ventral, from the ganglion on the ninth nerve. There are no perceptible ascending and descending divisions of these root fibers within the brain at this stage.

2. *The general cutaneous component.* The jugular ganglion holds about the same position relative to the other ganglia as in the younger stage, although there is now no apparent connection with the skin in its more caudal portion. It does connect with the skin, however, at a point near the second post-auditory lateral line primordium. This strand of cells is in very close association with the ganglionic projection to this primordium. It forms an incipient *r. auricularis vagi*.

3. *The visceral sensory component.* The epibranchial placode over the first branchial pouch is now massive and has a broad connection with the visceral ganglion of the ninth nerve (fig. 2, *G.Vis.IX*). It is now contributing cells, apparently, to the ganglion. The latter extends along the lateral line division of the nerve to the brain. Its attachment to the brain is shown in figures 30 and 31 (*R.Vis.IX*).

A projection of the visceral ganglion of the vagus still connects with a placode-like mass of cells situated over the second branchial pouch. The larger portion of the ganglion projects caudad and ventrad towards the other visceral pouches, near which it ends in indefinite clusters of cells. A slender strand of indifferent cells reaches from the ganglion to the brain, in which there may be a few root fibers (fig. 32, *R.X*). In figure 32 the strand of cells which represents the root (*G.X*), extends caudad between the brain and the myotome (*M*). Here a single root fiber appears. This may, however, be a motor fiber. No root fibers can be positively recognized as arising from cells in the ganglion.

C. THE COILED-REACTION STAGE

1. *The lateral line component.* The lateral line ganglion of the ninth nerve still reaches to the skin, but a relatively large strand of fibers without sheath cells now spreads across the primordium. This is an incipient *r. supratemporalis IX*. The ganglion has

enlarged greatly and stretches along the root till it comes into close relation with the lateral line root of the vagus (fig. 34). There is, then, a considerable advance made towards the consolidation of all the postauditory lateral line ganglia into a common mass (compare Goghill, '02, figs. 1 and 2). The ganglionic projection, however, which reaches out on the r. auricularis vagi now amounts practically to a distinct ganglion with its own root connection with the main lateral line root of the vagus. In the terminal portion of this smaller ganglion fibers connect with the primordium, but this nerve does not seem to be quite as well developed as the r. supratemporalis. It is a question whether these two nerves innervate a common primordium (Paper I, fig. 58) or whether there may not be two primordia here separated by an ectodermal thickening to which the jugular ganglion connects.

The primordia of the trunk now appear in two distinct patches (Paper I, fig. 58, *LL.*). One of these lies opposite the third and fourth myotomes and the other opposite the seventh to the tenth myotomes. To both of these the main lateral line ganglion of the vagus sends nerves, which separate from each other a short distance from the ganglion.

Figures 42 to 46 represent five successive serial sections which show the lateral line roots entering the brain. There are still two divisions of the roots, a dorsal and ventral, marked, respectively, *a* and *b*. Each division forms an ascending and a descending tract, the longitudinal extent of which is shown in figure 5.

2. *The general cutaneous component.* The jugular ganglion still lies on the lateral aspect of the proximal portion of the lateral line ganglion of the vagus as an ill-defined group of cells. It reaches the skin as a ganglion rather than as a nerve, in close relation with the small lateral line ganglion already mentioned. Its point of contact with the skin seems to be between the lateral line primordium innervated by the latter ganglion and that innervated by the r. supratemporalis. It projects also caudad in loose relation with the visceral ganglion, but its relations in this region are very indefinite. A root-like projection of the ganglion

passes ventrally of the lateralis ganglion and through the V-shaped cleft of the first myotome, but no fibers can be traced from this region into the brain.

3. *The visceral sensory component.* The visceral ganglion of the ninth nerve has enlarged greatly since the last stage, in which it appeared to be receiving cells from the epibranchial placode. It now touches the auditory vesicle (fig. 3, *G.Vis.IX*). Distally, however, it still reaches to the entoderm, where it has a very indefinite border. Throughout its distal portion it shows two rather distinct portions, the more dorsal of which projects the farther over the entoderm. No nerve trunks are yet to be found arising from this ganglion. It has a root, however, which enters the brain as a relatively strong bundle of fibers on the ventral aspect of the lateral line roots. This relation is shown in figures 44 and 45 (*R.Vis.IX*). The fasciculus solitarius (*Fas.Sol.*) is seen in figure 42 rostrally of the root. Caudally of the root it appears in figures 45 and 46, where it is larger. The most of the visceral sensory root fibers must, therefore, turn caudad in the fasciculus (fig. 5).

The visceral ganglion of the vagus still reaches out to the branchial pouches. No peripheral nerves yet arise from it. Its root enters the brain as shown in figures 47 to 49. Figure 47 shows ascending vagus fibers just ventrally of the glossopharyngeal portion of the fasciculus solitarius (*X*). Figure 49 shows that the fasciculus solitarius (*Fas.Sol.*) is smaller on the caudal side of the root. It is apparent from this that the visceral sensory fibers are directed chiefly cephalad in the fasciculus at this time. The fasciculus solitarius extends only a short distance caudad of the root (fig. 5, *R.Vis.X*).

D. THE EARLY SWIMMING STAGE

1. *The lateral line component.* In addition to the lateral line primordia of the trunk there are now two distinct postauditory primordia, the one innervated by the r. supratemporalis, the other by the lateralis component of the r. auricularis vagi (fig. 4; Paper I, fig. 59). The lateral line can be traced clearly as far

caudad as the seventeenth myotome, almost the full extent of the primordia. Fibers reach the primordium of the inferior line, also. The small lateral line ganglion which is associated with the r. auricularis vagi still stands out distinctly. The root fibers from the ganglia on the vagus pass to the brain on the dorsal surface of the root of the ninth nerve. The root fibers have extended their tracts as indicated in figure 6 (*L.L.IX.X Asc., Des.*). The relation of the root fibers to the tracts is shown in figures 62 to 65. The roots here are very compact bundles of fibers. There is no apparent interchange of fibers between the roots from the different ganglia. The dorsal tract seems to represent the vagus ganglion and the ventral tract the glosso-pharyngeal ganglion. The ventral tract runs rostrad in very close relation to the fasciculus solitarius and at the level of the middle of the auditory vesicle takes a position ventrally of the lateral line tracts of the facial nerve (fig. 61, *L.L.IX.X.b*).

2. *The general cutaneous component.* The jugular ganglion holds about the same position relative to the other ganglia as in the earlier stages. Only in favorable preparations can it be distinguished from the visceral ganglion, over which it lies in the form of a rather thin cap. It gives rise to the r. auricularis vagi, which is still a very short nerve. The exact distribution of the fibers can not be made out. General cutaneous fibers join also the projections of the visceral ganglion which reach out to the second and third branchial pouches. It has been impossible to demonstrate fibers from the jugular ganglion to the external gills, to the skin around the base of the gills or to the ventral surfaces of this part of the head. The cutaneous innervation, so far as the ganglionic system is concerned, is in a much lower state of development in the postauditory region than it is in the pre-auditory.

The root of the jugular ganglion now reaches the brain, passing across the dorsal surface of the visceral root from the rostral to the caudal side (fig. 4, *R.G.Jug.*). It enters the brain at a slightly more ventral level than does the visceral root, as shown in figures 67 to 69 (*R.G.Jug.*, fig. 69). This root is continuous from the ganglion to the brain surface with the spinal accessory

root (fig. 4, *R.XI*), which in this stage extends caudad to the level of the third myotome (compare Coghill '02, fig. 1, root *X4,a,b,c,d*). The latter root is probably largely motor in the early swimming stage, although cells that look like neuroblasts occasionally occur in it. The root of the jugular ganglion enters the descending trigeminal tract, as shown in figure 6. The diffuse nature of the tract in this region is illustrated in figures 66 to 70 (*Tr.Des.V*).

3. *The visceral sensory component.* The end of the glosso-pharyngeal ganglion still rests upon the entoderm, but there is now a postbranchial nerve arising from it which extends ventrad behind the pouch and runs a considerable distance rostrad in the primordium of the first branchial bar (fig. 4, *G.Vis.IX,g*). This is the ramus lingualis.

No fibers can be traced from this nerve to the skin. Neither do fibers pass from it into the entoderm. Nevertheless it must be primarily visceral sensory, although it may at this time contain motor fibers also. The nerve adheres in its course very closely to muscle primordia, just as do the r. mandibularis V, and the r. hyomandibularis VII of this age.

The root of this nerve still enters the brain in close contact with the ventral surface of the lateral line component (figs. 4 63, 64). Figures 62 and 65, respectively, show the condition just rostrally and caudally of the entrance of the root fibers, which join the fasciculus solitarius (*Fas.Sol.*).

The projections of the vagus ganglion have not yet completely lost their connection with the entoderm of the first and second branchial pouches, while the caudal portion of the ganglion frays out into loose cells over the branchial region. The first n. branchialis vagi passes some distance into the primordium of the second gill arch behind the corresponding pouch, but it is not as well developed as the r. lingualis of the glossopharyngeus (fig. 4, *G.Vis.X,h*). No other nerves can be found arising from the ganglion at this time. There appear to be no pharyngeal or prebranchial divisions of any of the postauditory branchial nerves in the early swimming stage.

The root of the vagus ganglion is distinctly more developed than in the earlier stage. As it approaches the brain it turns distinctly dorsad from beneath the general cutaneous root (fig. 3, 67 to 69, *R.Vis.X*). These root fibers must have a very short course within the brain, for the fasciculus solitarius (*Fas.Sol.*) diminishes considerably in thickness within the space of $20\ \mu$ from the root entrance, as shown in figure 66. A few fibers of the fasciculus, however, extend caudad for some distance (fig. 6). Dorsally of the fasciculus solitarius are found a few fibers of the sensory ascending tract from the spinal cord (figs. 66, 67, 68, 70, *DT*); and ventrally of it is the descending sensory tract of the trigeminus. At no place has the fasciculus solitarius become displaced from its earliest position beneath the external limiting membrane to the deeper position which characterizes the adult.

4. *The eye and optic nerve*

While the retina is recognized as essentially a part of the brain and the optic nerve as morphologically a tract of the central nervous system, the function of the eye as an exteroceptor requires that it be treated here with the afferent system. The structure of the eye at the four successive stages of development under consideration has been illustrated by figures 72 (non-motile), 74 (early flexure), 76 (coiled-reaction), and 78 (early swimming). These are all taken from transverse sections of embryos at the level which shows the greatest extent of connection of the lens with the skin, or as nearly as could be determined, through corresponding points in the eye.

In the non-motile stage (fig. 72) the pigmented layer is thick at the margins but soon tapers off into a relatively thin, simple epithelium, which has not yet acquired a high degree of pigmentation. It fits down closely upon the retina, excepting in the ventral portion where clefts may occur here and there beneath it. The central ends of the retinal cells have well defined boundaries, and have a considerable pigment deposited about them. Mitosis is going on rapidly throughout the entire peripheral (ventricular, with reference to the brain) border next to the pigment layer. The lens at this stage is simply a discoid thickening

of the epithelium, which fits into the optic cup. Its margin conforms closely to that of the retina but there is a narrow space between them. In the ventral portion of this space mesenchyme cells occur.

In the early flexure stage (fig. 74) the pigment layer has become thinner, particularly at the margins, and its pigmentation has increased considerably. The optic cup has greatly deepened. The cell boundaries along the central border of the retina have become less distinct and cells occur there which suggest the nature of neuroblasts. Mitotic figures are still abundant in the central portion of the ventricular margin of the retina. The lens has acquired the form of a hemisphere, or slightly in advance of this, since the line of contact with the skin does not equal the diameter of the lens. Mitosis is going on rapidly in the central portion of the lens and there is a faint suggestion of a cavity in it. The space between the lens and the retina has greatly increased, owing, apparently, to the increased curvature of the retina, and mesenchyme cells have pushed in almost to the center of this space (fig. 74, *Mes.*). Mesenchyma is invading the space, also, from dorsad.

The retina of the coiled-reaction stage (fig. 76) has rounded up into more nearly a spherical form. Cells which appear to be neuroblasts are now conspicuous in the middle region of the inner margin. The cells along the whole outer border (ventricular surface) are still proliferating rapidly by mitosis. While the lens is almost free from the skin, there is still a small area of close contact with it. A distinct cavity has appeared in the lens, on the outer side of which the epithelium is much the thicker. The skin over the lens is still thick, and mitosis continues in the lens.

In embryos of the early swimming stage (fig. 78) the skin over the lens has become reduced to a thin epithelium, which, however, is still pigmented in a considerable degree. The lens, though in contact with the skin, is perfectly detached from it. The cavity in it has increased greatly in size, and the inner epithelium is now much the thicker. The cells here are long and columnar and their borders well defined. Mesenchyma extends

into the space between the skin and the retina, practically into contact with the lens both dorsally and ventrally. The pigment layer of the retina has now become highly pigmented, so that most of its nuclei are obscured from view. Mitosis is still going on in the middle of the outer layer of the retina, but it appears to be more abundant towards the margins. In the inner layer of the retina there now occur many ganglion cells, particularly in the middle region. Good silver impregnations show that the axones of these cells form an optic nerve which extends along the optic stalk into the brain, where they enter the most caudal portion of the chiasma ridge. The form and position of these cells are illustrated in figure 71. The axone of this cell passes into very close relation with the internal limiting membrane of the retina, where it mingles with other axones. Nothing can be made out concerning dendritic processes of these cells. They appear still to be unipolar.

According to favorable silver impregnations of both *A. punctatum* and *A. microstomum* Cope, these optic fibers form a chiasma which is in intimate relation with postoptic commissure at this time. The optic fibers, being more deeply impregnated than the other fibers of the commissure, may be followed clearly across the middle line of the brain. They appear to be a constant feature of the brain of the early swimming embryo, though they do not stand out clearly in stained preparation as they do in silver impregnations.

In the same preparations which demonstrate the ganglion cells and their fibers clearly nothing of a nervous nature can be seen deeper in the retina. There are some round nuclei which suggest neuroblasts destined to become bipolar cells; but the layer of rods and cones seems very embryonic, since, as mentioned above, mitosis is still going on in its central region and no well defined cellular organization appears there.

5. The olfactory organ and nerve

In the non-motile stage the olfactory organ consists of a greatly thickened patch of ectoderm which has externally a very slight concavity. It touches the brain only lightly in its dorso-caudal

region but not in the region from which the olfactory nerve of later stages arises. In the early flexure stage the external concavity has deepened slightly. The cells of the olfactory epithelium have become highly columnar and heavily pigmented peripherally. The organ has come into rather close contact with the brain in the rostral region, but no direct connection can be made out between them. In the coiled-reaction stage the external concavity has become a shallow pit with well defined margins, but the depth is not equal to the diameter of the aperture. There is now very intimate contact between the olfactory epithelium and the brain at the point where the olfactory nerve is about to appear, but no fibers have been observed entering the brain in this stage. In the transition from this to the early swimming stage the olfactory pit has deepened considerably but has not yet dilated perceptibly within the nares. The olfactory nerve has now become well established. It arises from near the rostral margin of the olfactory epithelium, where the latter is in actual contact with the brain. Silver impregnations indicate that the fibers arise from neurones situated near the base of the nerve, though the impregnations of the cell bodies has been very imperfect and unsatisfactory. In frontal sections (parallel with the longitudinal axis of the embryo) the fibers appear running both caudad and rostrad within the brain into a zone of highly differentiated neurones which, presumably, are the mitral cells. There is a cell-free area here which obviously represents the glomerular zone.

6. Summary

A. THE NON-MOTILE STAGE

The ophthalmic and Gasserian ganglia are both connected with the skin at their distal ends (figs. 1, 7 to 12). No fibers can be clearly traced from them to the skin. Their root fibers, however, reach the brain. Large cells, having the characteristics of the Rohon-Beard cells of the spinal cord, situated near the entrance of the trigeminal root, send processes out into this nerve (figs. 20, 21). The general cutaneous ganglion of the vagus (jugular)

is very indefinite in outline and has neither peripheral nor root fibers arising from it. The lateral line ganglia all have root fibers connecting with the brain. Their peripheral ends reach to the corresponding primordia of the lateral lines, excepting in the case of the ganglion of the hyomandibular system, the relation of which to the skin is very indefinite. The auditory vesicle (fig. 73) is continuous with the skin and with the auditory ganglion. A barely perceptible auditory root connects with the brain. The visceral sensory system is represented by the geniculate ganglion, the glossopharyngeal and vagus. All of these connect with thickened patches of ectoderm which are associated with the visceral pouches. The geniculate has a very small root which reaches the brain. A strand of cells connects the glossopharyngeal ganglion with the brain but no fibrillar elements appear in this. The vagus ganglion has no root. The structure of the eye at this stage is illustrated in figure 72. The olfactory epithelium has barely begun to invaginate and does not yet touch the brain at the point of future origin of the olfactory nerve.

B. THE EARLY FLEXURE STAGE

The ganglia of the trigeminal nerve have severed their connection with the skin and now have peripheral fibers which connect obscurely with the skin (fig. 2). The root of the nerve forms a very short ascending tract and a descending tract which, augmented by neurones of the second order, extends to the level of the auditory vesicle (fig. 22). The jugular ganglion of the vagus has a very indefinite outline. It connects directly with the overlying skin but has no perceptible peripheral or root fibers. The ganglia of the acustico-lateral system have acquired greater definiteness of outline and compactness of structure. They now send fibers to the various primordia, with which they are still in contact. Their root fibers enter the brain distinctly. The auditory vesicle has become detached from the skin and from the auditory ganglion (fig. 75). The visceral sensory ganglia are still connected with the epibranchial placodes and no peripheral fibers arise from them. The root fibers of the geniculate

and glossopharyngeal ganglia enter the brain, but the vagus ganglion has no root. The optic cup has deepened greatly and the lens projects deeply into its cavity. The invagination of the olfactory epithelium is more marked but there is not yet an olfactory nerve.

C. THE COILED-REACTION STAGE

The distribution of the trigeminal fibers to the skin is much more obvious than in the earlier stage, the mesial terminal branch of the *r. ophthalmicus profundus* being well defined. A descending trigeminal tract of a composite nature extends to the spinal levels (figs. 3, 5). The giant ganglion cell component of the trigeminus is now particularly clear (figs. 34 to 37). The outline of the jugular ganglion is still indefinite and no perceptible fibers leave the ganglion. The lateral line primordia have become much extended and a mandibular line makes its appearance. Lateral line nerves follow along the primordia closely attached to the skin, although no fibers have been traced in this stage to the mandibular line. All of the lateral line roots form ascending and descending tracts in the brain, and the auditory root forms a short descending tract (fig. 5). The auditory vesicle of this stage is illustrated in figure 77. The geniculate ganglion is still united with its epibranchial placode, but from it emerge several fibers which project ventrad behind the spiracular pouch as an incipient *r. alveolaris*. The root fibers from this ganglion form the fasciculus solitarius which runs caudad immediately beneath the external limiting membrane to the level of the glossopharyngeal root which enters it. Peripherally the glossopharyngeal ganglion connects with its epibranchial placode. The vagus ganglion now has a small root which joins the fasciculus solitarius. Distally this ganglion connects with the epibranchial placodes, but gives rise to no peripheral fibers. The condition of the eye is shown in figure 76. The olfactory epithelium is now in close contact with the brain, but olfactory root fibers do not certainly enter the brain in this stage.

D. THE EARLY SWIMMING STAGE

Of the general cutaneous system the lateral and mesial terminal branches of the r. ophthalmicus profundus, the r. maxillaris, r. mandibularis with a nerve to the balancer, and a nerve from the ophthalmic ganglion directly to the overlying skin are well defined (fig. 4). The r. auricularis vagi is perceptible but its distribution is uncertain. The root fibers of the trigeminus now extend in the descending tract to about the level of the root of the vagus nerve, and this tract is joined by the root of the vagus ganglion (fig. 5). The giant ganglion cell component of the trigeminal nerve is still present, but its exact distribution through the nerve can not be determined. The hyomandibular lateral line primordium is now continuous with the other pre-auditory primordia and is innervated by the r. hyomandibularis VII. The other lateral line primordia are now innervated practically throughout their entire extent, the lateral line of the trunk extending into the level of the seventeenth myotome. The extent of the ascending and descending tracts of the lateral line roots is shown in figure 6, where the extent of the descending auditory fibers is also figured. The visceral sensory component is now represented by a well developed r. alveolaris VII, r. lingualis IX, and a less developed first r. branchialis vagi. There are no prebranchial or pharyngeal branches of the postauditory branchial nerves. The vagus root now connects with the brain and contributes fibers to the fasciculus solitarius. In the retina mitosis is still going on rapidly in the middle of the external layer, while in the internal layer ganglion cells are already developed which send their axones along the optic stalk into the brain. They decussate in the most rostral portion of the post-optic commissure. Only a suggestion appears of neuroblasts in the region of the bipolar cell layer. The olfactory nerve is well established and its fibers distribute themselves in a very definite glomerular zone of the olfactory lobe.

II. THE PHYSIOLOGICAL PART

1. *Response to tactile stimulation*

Early in my study of the responses of these embryos to tactile stimulation it became apparent to me that the skin of the pre-auditory region was less sensitive than was that of the post-auditory region. For conclusive evidence upon this point, however, I am indebted to one of my graduate students, Miss Ruth Orcutt, who in March, 1911 carried out a series of experiments, two of which I introduce here as typical of her results.

Ten specimens were selected which were approaching very near to the typical coiled-reaction stage, their movements being not quite extensive enough to be typical. These were touched with a hair one after the other in regular order in the following manner: At each trial an embryo was at first touched in the region of the eye. If this stimulus produced a response, the next embryo of the series was touched; if it did not produce response, the same embryo was, after an interval, touched over the post-auditory region in the territory of innervation by the vagus nerve. If response occurred, the next embryo was tested; if not, the same embryo was then touched over the myotomes of the cervical region, the territory of the Rohon-Beard cells of the spinal cord. The ten embryos were worked over in regular order in this manner till one hundred forty-three responses occurred. Of these five were in response to touch on the preauditory region; 66, to stimulation of the territory of the vagus nerve; and 72, to stimulation of spinal territory strictly.

Another group of embryos of a distinctly more advanced stage, being in the advanced coiled-reaction stage, were tested in the same manner. These gave 94 responses, of which 12 were in response to stimulation of the trigeminal region; 41, to stimulation of the territory of the vagus; 41, to stimulation of the spinal region.

These and other experiments upon this question give unequivocal evidence that stimuli from the spinal field reach the motor centers with much greater facility and certainty than do stimuli

from the territory of the vagus nerve; and that, as compared with stimuli from these areas, stimuli very rarely reach the motor centers from the trigeminal field in embryos of the typical coiled reaction stage. At the time these experiments upon this subject were made we had no exact information upon the nature of the innervation of the regions under consideration. The anatomical part of this paper, however, shows that the trigeminal nerve, throughout the whole period of development up to the early swimming stage, runs far ahead of the general cutaneous component of the vagus in the development of both peripheral and root fibers. The relative accessibility of motor centers, therefore, to stimuli from trigeminal and vagus territories can not depend upon the relative development of these nerves. The certainty of responses, when the stimuli are applied to the spinal region, obviously depends upon the perfection of the giant ganglion cell system of afferent neurones and their direct access to motor centers (Paper I). The neurones of this system occur as far rostrad as the second myotome, which is essentially within the limits of the medulla oblongata. They may, indeed, occur here and there farther cephalad as shown in this paper. Their peripheral fibers, therefore, probably invade the region which was regarded in the above mentioned experiments as belonging to the vagus, but in a less efficient manner than in the trunk, so that response is not as certain from stimulation here as it is from stimulation farther caudad. The response to stimulation of the whole postauditory territory and trunk is in all probability effected through the giant ganglion cells system in the coiled-reaction stage. And since we now recognize cells of this type in the trigeminal nerve, such responses as occur to stimulation of this region in the earlier periods may take place through this system also. Granting, however, that all the giant ganglion cells in the trigeminus are distributed to the skin rather than to the muscle primordia, the innervation of the preauditory region by this system must be very sparse as compared with that of the postauditory and trunk regions, for there are only a few giant ganglion cells in the vicinity of the trigeminal root. Such sparseness of innervation, upon the hypothesis that the gan-

glionic portion of the trigeminus is not functionally connected with the motor centers, might explain the low degree of responsiveness to stimuli in this region. On the other hand, if the responses are effected through the ganglionic system of the trigeminus their infrequency in the coiled-reaction stage must depend upon the limitations of the zone of influence of the root fibers within the brain, for the peripheral distribution of the nerve at this time is very well developed.

As a factor in this problem the descending trigeminal tract naturally suggests itself. This tract has been described in the first part of the paper as being of a composite nature—made up of processes of neurones of the first and second, and possibly higher orders, in varying proportions according to the region. The root fibers of the trigeminal ganglia run some distance caudad in the coiled-reaction stage—probably almost to the level of the auditory vesicle. Beyond this the tract, now extending into the spinal region, consists of processes from central neurones along the course. If, then, stimuli reach the motor centers in the lower regions of the medulla oblongata through the descending trigeminal tract they would have to traverse a series of relatively short neurones with intercalated synapses after leaving the trigeminal conductors. Such a conduction path would certainly introduce relatively great resistance if it did not block the stimuli altogether. This is, in all probability, the really significant factor in determining the behavior of embryos of the coiled-reaction stage to tactile stimulation of the preauditory region.

In the early swimming stage the condition is quite different. The trigeminal fibers now reach almost or quite to the level of the vagus root, that is, into the immediate vicinity of the centers that dominate the whole motor mechanism. Accordingly, response to stimulation of the trigeminal region has become prompt and regular. The data at hand, therefore, afford very good evidence that such a descending trigeminal tract as occurs in the early flexure stage (fig. 22) is at best a very inefficient conductor; and that before response can become prompt and constant the root fibers of the nerve must reach directly into the approximate levels of the motor centers. We have in this con-

dition, then, the significance of the rapid growth of the descending trigeminal tract between the coiled-reaction and early swimming stages, for there is very close correlation between the development of the power of locomotion and the introduction of the most anterior sensory field of the organism into direct connection with the motor centers so that it becomes effective in determining the direction of locomotion.

The significance of this correlation in the growth of the motor and sensory systems finds a striking demonstration in the reactions of embryos of advanced swimming stage which have been transected in the upper portion of the trunk (Paper I, p. 200). This operation cuts out both the exteroceptive and proprioceptive stimuli from the greater portion of the trunk and leaves the motor centers more exclusively to the influence of the cutaneous field of the head. In head pieces of this kind, which have just enough of the trunk musculature attached to give unequivocal responses, the movements are almost universally away from the side touched. It is obvious, therefore, that this avoiding form of response to stimuli about the anterior end of the embryo is a basic thing in the orientation of the swimming animal to its environment; and, judged by the anatomical results of this paper, its perfection and efficiency depend upon the growth of the trigeminal root fibers into proximity with the motor centers.

In the above experiments upon the relative irritability of different areas it was observed that the regions under consideration differed materially with reference to the nature of the response they evoke. In the first set, three of the five responses to stimulation of the trigeminal area were away from the side touched, whereas 62 of the 66 responses to stimulation of the vagus field were away from the side touched, as were all of the 72 responses to stimulation of the spinal field. In the second experiment mentioned the embryos were considerably older and all of the 94 responses were away from the side touched excepting two of the twelve responses to stimulation of the trigeminal area. These data are in accord with my general experience with these embryos, namely, that with increased responsiveness there is increased purity of type in response. In other words, embryos

that are not readily excited to motor response are much more likely to move towards the side touched than are embryos that are easily excited to movement.

The conclusion of the last paragraph raises a question which has been mentioned in an earlier paper ('09, p. 252) as to whether movement away from the side touched is a fundamentally primary thing in behavior or a secondary thing, derived by a process of selection out of a diffuse or irregular type of response. From the conclusion reached concerning the structural basis for prompt and certain response to stimulation of the trigeminal field, it might be inferred that this problem also hangs directly and solely upon the degree of development of the peripheral neurones in their relation to the motor centers. In other words, if a stimulus must pass through such a descending trigeminal tract as is shown in figure 22, representing the early flexure stage, there is a larger element of chance or accident in the direction of the response than there would be if the stimulus traveled into the immediate vicinity of the motor nuclei through the peripheral neurones. This would be an attractive hypothesis, but, to my mind, it is only of secondary consideration. When, for instance, there is a high degree of responsiveness (a low threshold of stimulation for the entire reflex arc concerned) a stimulus such as is employed experimentally would act in a much more localized or restricted area than it would when there is a low degree of responsiveness. Under the latter condition the stimulus would probably be transmitted to relatively distant receptors, possibly even to the opposite side of the embryo and, finding here access to an arc of lower threshold, it would excite a response that apparently violates the regular law of behavior. This explanation gains credence from Hooker's experiments ('11) which show that amphibian embryos of the ages under consideration are very sensitive to stretching of the skin by pressure upon relatively distant points. In fact his experiments show conclusively that in order that a tactile stimulus act locally in any appreciable degree in these embryos there must be a very low threshold of stimulation for the reflex arc as a whole. Undue resistance at any point in the arc would certainly allow the

stimulus to act on more distant receptors of arcs with, possibly, a lower resistance. The cause of irregularity in the direction of response when responsiveness is low is, therefore, in all probability to be found in the receptor system and adequate stimulation of an arc as a whole rather than in an hypothetical diffuse form of conduction through the central nervous system.

2. Response to chemical stimulation

The question of the existence of a true irritability to substances in solution as opposed to their destructive action on the skin has been discussed in Paper I. By spraying small jets of acid upon restricted regions of the embryo and by immersing head pieces of transected specimens in acid it has been shown that the skin of the head is like that of the trunk in its sensitiveness to the action of acid. It appears, therefore, that the definitive, ganglionic nerves of the ages under consideration do not differ from the giant ganglion cell system in respect to chemical stimulation.

3. Response to light

During the season of 1910 I performed a series of experiments upon the reaction of larvae and embryos of *A. opacum* and *A. punctatum* to light. Sunlight, thrown from a mirror through a water jacket and condenser, was employed in such a way as to bring to bear very high illumination upon sharply restricted regions of the animals. As a check on the adequacy of the stimulus a larva of *A. opacum* was used which was about ten days beyond the age when swimming begins. When intense illumination was brought to bear over the head and eyes of this larva responses were fairly prompt, as the following figures show, the reaction times being indicated in seconds, with the interval between one response and the next stimulus indicated in parentheses: 5, (15); 10, (15); 8, (20); 8, (30); 8, (20); 3, (30); 8, (30); 7, (30); 8, (30); 8. The responses were always quick, total body movements and swimming away from the stimulus, slightly to one side rather than straight forward from the position when stimu-

lated. Immediately following this series of responses the larva lay with its trunk and tail only in the highly illuminated field for five minutes without movement, excepting one small movement of the head. In a fainter light, but with illumination sufficiently strong to cast a distinct shadow upon the dark surface on which the larva rested, it lay for ten minutes longer motionless. The responses recorded above, therefore, must have been to stimulation of the eyes and not to an action of the light upon the skin; and the irritability of the skin to light in this relatively late stage must be negligible.

The methods of the above experiment were applied to five embryos of *A. punctatum* which had almost but not quite reached the swimming stage, but the results gave no evidence of irritability to light. Four specimens which had reached the early swimming stage, however, gave the following results (the time of application of the stimulus being indicated in seconds, the plus sign indicating a reaction after the time indicated, the minus sign indicating no reaction):

- Specimen a. 20 +, 20 +, 10 +, 60 -, 30 +, 30 +, 10 +.
- Specimen b. 60 -, 20 +, 120 +, 10 +, 60 +, 10 +, 60 -.
- Specimen c. 60 -, 70 +, 60 -, 60 -, 20 +, 15 +.
- Specimen d. 60 -, 45 +, 50 +, 60 -, 60 -, 60 -, 60 -.

The specimens were stimulated one after the other so that there was a relatively long interval between successive stimuli applied to the same specimen. The illumination during this period may not have been absolutely constant but it was at all times very brilliant. As embryos of this age only rarely move without some form of external stimulation, it appears that the responses observed in this experiment were due to the stimulation of the retina by light, but the threshold of stimulation is exceedingly high. The shortest reaction time was ten seconds, as compared with three seconds in the older larva; whereas one specimen lay in one trial for two minutes before responding and in two other trials gave the shortest reaction time.

Another set of specimens of the same age were tested in the same manner with the following results:

Specimen e.	60 - , 60 - , 60 - , 60 - , 60 - , 60 - , 60 - , 60 - , 60 - .
Specimen f.	60 - , 60 - , 35 + , 25 + , 60 - , 55 + , 60 - , 60 - , 25 + , 30 + .
Specimen g.	60 - , 60 - , 30 + , 60 - , 60 - , 45 - , 50 + , 60 + , 60 + , 35 + .
Specimen h.	55 + , 60 - , 60 - , 60 - , 60 + , 60 - , 60 - , 60 - , 53 + , 60 - .

In brief, specimen *e* gives no evidence of irritability to light. *f* and *g* show noteworthy evidence of it, while the other three show slight evidence of it. It may be said, therefore, that at the time swimming begins the retina is just beginning to be irritable to high illumination.

The anatomical explanation for these results will not be fully before us till the central nervous system is better understood, but it is important to note here that it is just at this stage that the optic nerve makes its connection with the brain and that the retina is in a very embryonic condition in its outer layers. In the light of these reactions interest attaches to this order of development in the retina. The ganglion cells, it has been noted, are well differentiated and their fibers decussate in the incipient chiasma. These neurones have as high a degree of differentiation as have other neurones that are known to be in the functional condition. The inference, therefore, is that the high and variable threshold of the retina at this stage is due to the embryonic condition of the more peripheral elements in the reflex circuit. On the other hand, it is interesting to know that with the layer of rods and cones and the bipolar cell layer in such an embryonic condition there could be any optic reflexes stimulated. One almost questions whether the ganglion cells themselves may not possess at this time a certain degree of irritability to light. In the early swimming stage, as described in the anatomical part of the paper, mitosis is still going on in the central zone of the layer of rods and cones and only suggestions of neuroblasts can be detected in the region of the layer of bipolar cells. It seems incredible that specialized photic receptors and conduction paths can be already established in such embryonic structures. Upon the basis of studies which are now in progress on the central paths of the optic reflexes it will be necessary to return to more exhaustive cytological study of the retina and to an amplification of

the experimental evidence of the development of function in the organ.

4. Response to olfactory stimulation

In the anatomical part of the paper it was noted that the olfactory nerve is well established in the early swimming stage. Preliminary experiments were made in 1912 to discover whether olfactory stimuli in any way stimulate movement. Pellets of various substances were enclosed in gauze and placed in the center of flat dishes about 9 by 13 cm. In these dishes embryos somewhat older than the early swimming stage were distributed in cistern water. Pellets of pure gauze were used as checks. In other pellets were placed, egg masses, fresh algae and débris from aquaria which emitted a strong odor. The embryos in all the dishes were frequently agitated to discover whether the olfactory organ had any influence in directing movements stimulated from other causes. Over thirty embryos in the different test dishes and the same number in the check dish were kept under observation for three hours without the manifestation of any tendency on the part of the specimens to collect in the vicinity of the odiferous substance. A number of experiments of this kind gave no further suggestion of olfactory reflexes. Experiments of this kind, however, should be carried further to determine whether other substances may not be found which would stimulate olfactory reactions, either directly or by reinforcing or inhibiting other responses. It has been noted however, that, although olfactory neurones have grown into the brain in considerable numbers, the olfactory epithelium is very poorly differentiated in the ages under consideration.

5. The auditory and lateral line organs

The relative development of the lateral line system of organs and nerves during this period arouses inquiry concerning their function. In the postauditory region this system develops distinctly in advance of the general cutaneous component in both its central and its peripheral relations. Even in the preauditory

region, where the general cutaneous system is more precocious, the connection of the lateral line nerves with the skin becomes readily recognizable earlier than in the case of the general cutaneous nerves, and throughout the period under consideration the lateral line nerves have the more intimate and the more extensive contact with the skin. Judged upon the anatomical basis, therefore, the lateral line system would certainly be held as playing a larger rôle in the life of the organism at this time than do the general cutaneous nerves. I have been unable, however, to discover what this function may be and have further experiments planned.

The auditory organ, being a simple dilated vesicle, gives no evidence of being a mechanism that would be adapted as a receptor to the function of equilibration, so that the auditory nerve, which has established quite extensive relations within the brain and intimate contact with the vesicular epithelium in the early swimming stage probably does not enter into the reactions of the organism till a later period. As is well known, the embryos of this stage lie on the side while at rest and hold the upright position only during locomotion. The embryos which I select as representing the early swimming stage move upon the substratum, and their upright position during locomotion is probably the result of the lateral thrust of the head, which would tend to prevent them from settling down on either side till motion ceases.

6. Summary

a. The preauditory portion of the ganglionic general cutaneous system develops far in advance of the postauditory portion. The former becomes a factor in quick and certain response only about the time the embryo begins to swim, although response to preauditory stimulation occurs irregularly and infrequently for a considerable time before this.

b. The entrance of the receptor field of the trigeminal nerve into function as an important factor in the behavior is closely correlated with the extension of the root fibers of the trigeminal

nerve into the immediate vicinity of the motor centers in the lower portion of the medulla oblongata.

c. Such irregularity as appears early in development in the direction of movement relative to the side touched depends upon the condition that the point of adequate stimulus is not the point actually touched, by reason of the variability in the threshold of stimulation of reflex arcs as a whole, and not upon diffuse conduction through the nervous system. Reflex arcs are from the first definite and fixed during the period under consideration.

d. The afferent system of the head is like that of the trunk with reference to chemical stimulation.

e. A slight responsiveness to high illumination of the retina occurs about the time the animal begins to swim, and this is in close correlation with the development of the first fibers of the optic nerve into the brain. Owing to the exceedingly embryonic condition of the retina at this time this topic demands further investigation.

f. Although the olfactory nerve is well established at the end of the period under investigation there are no perceptible reactions to olfactory stimulation.

g. There is no available evidence that the auditory organ or lateral line nerves have any part in reactions, although the latter, judged upon the basis of its anatomical relations, would be regarded as more efficient than the ganglionic general cutaneous system.

h. The physiology of the eye, olfactory organ, auditory organ and lateral line organs require further investigation in connection with anatomical studies of the brain, which are now in progress.

III. DISCUSSION

It is the purpose to deal here with the work of other investigators which seems to have direct bearing upon the results of this paper.

1. Lateral line primordia, placodes and ectodermal thickenings

In the use of the terms 'lateral line primordia,' 'placodes' and 'ectodermal thickenings,' I am following Landacre and Conger in their work upon *Ameiurus* and *Lepidosteus* (Landacre, '10, '12; Landacre and Conger, '13). I wish, however, at the outset of this discussion, to emphasize the fact that my series of *Amblystoma* embryos is not adapted to a critical study of the histogenesis of these structures, for my specimens have been selected at relatively long intervals according to physiological stages and with reference to a problem that is not primarily one of histogenesis. It seems obvious, nevertheless, that there is a striking resemblance between *Amblystoma* and *Lepidosteus* (Landacre and Conger, '13) in the early processes of differentiation of sense organs and nerves.

While I have not undertaken to determine to what extent epibranchial placodes contribute to the formation of the various cranial ganglia, it seems certain that the visceral ganglia of the facial, glossopharyngeal and vagus nerves all receive masses of cells from this source, particularly during the middle and latter part of the period covered by my studies. This tardy differentiation of the visceral as compared with the somatic sensory ganglia is correlated with a late entrance of the visceral sensory field into the behavior, and is particularly noteworthy in connection with Landacre's observation that the relatively late differentiation of the epibranchial placodes in *Lepidosteus* as compared with *Ameiurus* is correlated with a late development of the taste buds in the former as compared with the latter, according to particular growth periods.

No lateral line placodes have been recognized in *Amblystoma* of my series, unless the ectodermal thickening which joins the auditory vesicle to the preauditory lateral line primordium be regarded as a vestige of a dorso-lateral placode. In section this thickening of the skin has the appearance of that which is formed in the early flexure stage over the ophthalmic ganglion where the latter has just become detached from the ectoderm. The significance of this attachment of the ophthalmic ganglion to

the skin does not seem to be understood. Landaere ('12) observed the same relations in *Lepidosteus*, but, apparently, did not satisfy himself concerning the meaning of it.

The ectodermal thickenings which Landaere and Conger have differentiated from the epibranchial placodes and the lateral line primordia certainly will be found also in *Amblystoma*, although my studies have not extended to the details of their relations. This subject deserves exhaustive study from material selected for the purpose. My object in touching upon this topic has been merely to bring the salient features in the differentiation of the nervous system which these structures represent into correlation with physiological processes in the organism as a whole. Limited as this study has been, however, I have been increasingly impressed with the results of Landacre's studies on this subject as fundamental to a broadly biological view of the development of the nervous system of the vertebrate head. These ectodermal thickenings and proliferations impress me not simply as vestiges of an ancestral metamerism or branchiomeres, but as centers of physiological processes which may be essential factors in the regulation of the growth of the nervous system. There is a field here for experimental studies, it seems to me, which might prove as illuminating as the well known observations upon the correlative development of the lens and the retina, and it is to be hoped that the purely embryological studies of these structures may be extended to other types of vertebrates.

2. The function of the auditory vesicle

Streeter ('06) has studied the development of the ear as correlated with the development of the function of equilibration in the tadpole of the frog. He found that shortly before tadpoles acquire the ability to maintain an upright position "the labyrinth consists of a closed epithelial sac incompletely subdivided into compartments and possessing differentiated nerve endings which are connected with the brain by the acoustic nerve and ganglion." The semicircular canals are "in the process of development, but are not completely pocketed off until after equilibration is already

established. Consequently the semicircular canals as such are not an essential factor in equilibration." He observed, further, that tadpoles from which he removed one auditory vesicle in the 3 mm. stage began, when 9 to 9.5 mm. long, to differ from normal tadpoles in the manner of swimming—the operated specimens having a tendency "to swim with the operated side under, and in the rolling movements around the long axis of the body it is from the operated side under to the opposite." Streeter concludes that it is at about this time that "functional union is normally established between the ear vesicle and the spinal cord reflex centers, upon which the individual is dependent for maintaining its position in free water." Estimated by Streeter's notes, this is about two days after the animals begin to swim, and, therefore, at a stage much beyond that which I call the early swimming stage in *Amblystoma*. This difference in age is expressed in the structure of the ear as well as in the efficiency of the motor system in locomotion, for the ear of the *Amblystoma* early swimming stage is a perfectly simple sac, except for the endolymphatic appendage—a much simpler organ than the ear of the frog tadpole when it acquires the power to swim upright in free water. Streeter notes that the auditory nerve is developed at this time, but does not describe its central relations in detail. While it would be interesting to know these central relations from the point of view of functional mechanism as a whole, Streeter's results seem to be conclusive on the question of the relation of the auditory organ as such to the function of equilibration, and they do not lead us to expect any functional union of the ear with the motor centers in embryos of *Amblystoma* of the early swimming stage.

3. The sensory nerve roots in relation to later development

The medulla oblongata of *Amblystoma* of lengths from 17 to 38 mm. has been recently described by Herrick ('14). Such larvae are practically adult so far as reactions are concerned and in the structure of the medulla oblongata Herrick considers that all the fundamental relations of the adult have been acquired.

A comparison of his descriptions with the anatomical results of this paper will show that the general plan of the sensory centers of the medulla oblongata is laid down by the growth inward of the root fibers of the peripheral nerves. The trigeminal and lateral line fibers have formed extensive ascending and descending tracts, and the auditory and geniculate ganglia have formed descending tracts of considerable length by the time the animal begins to swim. These tracts are all closely grouped and all lie immediately beneath the external limiting membranes, and constitute the substantia alba of the dorsal portion of the medulla oblongata. They lie embedded in the marginal zone of the substantia grisea, in which are neurones directed dorso-ventrad across the whole series of tracts of the various sensory modes. In the neurones of this group there can scarcely be any specificity of function with reference to the different sensory nerves (compare Herrick and Coghill '15). To this elementary plan the adult has added the longitudinal association tracts *a* and *b* of Kingsbury and Herrick, and an additional lateral line tract, while the fasciculus solitarius has become detached from the periphery and with its displacement inward the lateral line centers have developed greatly ventrad as well as dorsad.

Without knowledge of intermediate stages between the early swimming stage and the younger of Herrick's descriptions it is impossible to say just how the three root bundles of the lateral line VII of Herrick are derived from the two bundles of the younger stage. Since, however, the root bundles of the younger stage have the same relation to each other and to the root bundles of the postauditory system as do the two more ventral bundles of Herrick, and since the most dorsal bundle of Herrick has, in younger stages, smaller fibers than the other bundles, it is reasonable to infer that the most dorsal bundle of Herrick is a distinct and newly acquired tract, and not a detached portion of one or the other, or of both of the tracts of the younger stage.

In the relation of the several root bundles to particular ganglia Herrick finds that, in the case of the facial division, each of the tracts receives fibers from both the preauditory ganglia. This is a very difficult relation to determine in the young embryos,

for they must be studied under very high magnifications, and the roots come into very close relations with each other as they approach the brain. It seems unquestionable, however, that the more dorsal tract is derived chiefly from the dorsal ganglion (a) and that the ventral tract comes chiefly from the ventral ganglion (b). In the case of the postauditory group, the dorsal tract is derived chiefly from the vagus and the ventral, chiefly from the glossopharyngeal ganglion. It is impossible to say that there is no interchange of fibers between the roots of either set, but if such anastomosis does occur it must involve only a very few fibers.

If these lateral line tracts were each composed of fibers from one ganglion exclusively there would be grounds for assuming that they effect a certain degree of localization in the brain with reference to the particular areas with which they are severally connected. Herriek's observation of the mingling of the fibers of the different roots, however, makes it necessary to seek some other explanation for this early differentiation of the lateral line system within the brain. There is nothing in the sense organs to suggest that this has to do with different sensory modes. Its significance, therefore, must in some way have to do with the directness with which stimuli may pass to the motor centers. But that this mechanism as it is found in early swimming embryos could serve such a function seems incredible, particularly in view of the nature and arrangement of the neurones of the second order in the circuit. It is hoped that the study which is in progress on the association paths of the brain may offer something towards the solution of this problem.

In view of Herriek's observations of the bifurcation of the root fibers, it is of interest to note, further, that in the case of the trigeminal and lateral line nerves this takes place very early in development. In the case of the visceral sensory and auditory roots, however, there is little or no evidence of bifurcation of the fibers in the early swimming stage.

4. The development of function in the neurone and in the reflex arc

It has already been pointed out (Paper I) that the giant ganglion cells of the spinal cord on the one hand and the motor neurones on the other have their connections made with their respective end-organs for a considerable time before they become integral parts of a functional reflex arc, and that, for this reason, these neurones can not be used as a standard by which to judge the structural changes in the neurone that mark the beginning of nervous function. Now we find that the cranial afferent nerves also are in the earlier stages developed in a degree that seems out of proportion to the part that they can be demonstrated to have in reflexes. We have noted, for example, that, in the coiled reaction stage, the trigeminal nerve has a very general distribution to the skin and that it has root connection with the brain from a much earlier period, whereas it becomes an important factor in behavior only about the time the embryo begins to swim. The neurones of this nerve must for a considerable time receive stimuli and conduct impulses that are never discharged through any reflex arc to the effectors. The root fibers of the visceral sensory system also are well developed and the fasciculus solitarius is extensively developed for many days before the neurones of this system are subjected to physiological stimulation by the development of the mouth. None of these neurones, therefore, can give us a clue as to when they take on nervous functions.

Our observations upon the development of the optic nerve in relation to the function of the eye show that the same uncertainty is attached to the neurones of the second or higher order in the arc. In this case the retinal ganglion cells are highly differentiated and can, under very strong stimulation of the retina, become an integral part of a reflex arc at a time when the more peripheral sensory elements of the arc are in an exceedingly embryonic condition. The high threshold of the optic reflex must then be due to the undeveloped condition of the retina whereas the ganglion cells, optic nerve fibers, and the central neurones reaching caudad from the diencephalon to the lower

portion of the medulla oblongata must be capable of conducting stimuli for a considerable period before they can be excited by normal stimulation of the retina. The development of the olfactory nerve and centers suggests that the same principle applies to much of the central nervous system in early development. We have, then, within our knowledge of the development of reflexes and reflex arcs no rational basis for cytological studies of the differentiation of the neurone as correlated with the development of nervous function. It is hoped that the studies which are now in progress upon the central conduction paths will furnish such a basis for cytological investigations; and that the application of cytological methods to nerve cells of known physiological capacity may reveal the essential structural basis of conduction in the neurone.

5. The cranial nerves of Amphibia

Some points of interest concerning the morphology and development of the cranial nerves of Amphibia deserve mention here.

In my paper on the cranial nerves of *Amblystoma* ('02, p. 215, and figure 1) the lateralis ganglion which corresponds to VII *a* of this paper is described as closely fused with the ganglion of the ophthalmicus profundus, and the latter as very intimately related with the Gasserian ganglion. In the younger embryos of this study these ganglia are widely separated, but in the latter part of the period under investigation these ganglia, particularly the Gasserian and profundus, approach each other rapidly, and finally the Gasserian and profundus ganglia establish a wide contact, the lateralis ganglion VII *a* still standing apart from them. A similar change, though of less degree, occurs in these ganglia in *Rana pipiens*, according to Landacre and McClellan ('12), who describe the ganglion of the ophthalmicus profundus in the 8 mm. embryo as standing out "rather distinctly, indicating its definite character," and as being much more isolated in younger stages. In the early development, therefore, of both Anura and Urodela there is a process of consolidation going on between the various ganglia of the V + VII complex, a process

which goes farther in Anura than in Urodela, that is to say, till all the ganglia become fused into a common mass, whereas they become two masses in the Urodela.

This migration and fusion of ganglia involves not only a shifting of position relative to each other but also a general movement of the ganglia towards the brain along the path of their respective roots. There is also a shifting of the otocyst rostrad with reference to the point of entrance of the facial and auditory roots into the brain. Such a movement of the vesicle together with its expansion in growth, if carried far enough before chondrification of the auditory capsule sets in, might explain the fusion of all these preauditory ganglia into a common mass in Urodela; but this could not explain the migration caudad of the ganglion of the *ophthalmicus profundus*, for instance, as illustrated in figures 1 to 4. There is apparent no purely mechanical cause for such movement on the part of these ganglia.

A very similar migration and a consolidation of ganglia occur in the postauditory ganglionic complex as well. In adult *Amblystoma* there is a single postauditory ganglionic mass which can be resolved into its component ganglia only with difficulty (Coghill, '02, p. 231 and fig. 1). In the ages studied here (figs. 1 to 4), however, there are two widely separated ganglionic masses, one upon the glossopharyngeal and the other upon the vagus nerve. Changes very like this occur in Anura according to Landacre and McClellan, for they describe a rather marked distinctness of the different ganglia of the postauditory complex in 8 mm. embryos of *Rana pipiens*. The fusion of these ganglia may be accounted for by the dilation of the auditory vesicle in later development, and the consequent pushing caudad of the glossopharyngeal ganglia upon those of the vagus. Before this takes place, however, there is a perceptible migration of some of these ganglia along their respective roots towards the brain, although this is not as clear as the migration of the trigeminal ganglia. The chief differences, therefore, between the cranial ganglia of Anura and those of Urodela depend, apparently, upon two factors—an active migration of the various ganglia towards

the brain and pressure exerted by the dilation of the auditory vesicle in its growth.

In my discussion of the hyomandibular nerve of Amphibia ('02, pp. 264-271) I pointed out differences between the Anura and Urodela which then seemed to me to warrant the hypothesis that the ramus alveolaris VII of the latter was a pretrematic nerve. The essential basis for that hypothesis was the apparent prebranchial position of the nerve in relatively advanced larvae "anteriorly of the deep pharyngeal evagination which represents the embryonic spiracular cleft" ('02, p. 228). This position, however, must be secondary, for, as described in this paper, the first nerve that grows out from the geniculate ganglion enters the truncus hyomandibularis and passes behind the ectodermal-entodermal contact of the spiracular pouch. This position must be primary, as Emmel found it to be for the chorda tympani in the development of *Microtus* ('04), and the nerve must undergo very much the same kind of a shifting in position from an actual postbranchial position to an apparent prebranchial position just as the chorda tympani does in mammals, according to Emmel's descriptions. Contrary to my earlier interpretation, therefore, the r. alveolaris VII of Urodela should be regarded as homologous with the chorda tympani of mammals.

6. *Neurobiotaxis*

In numerous contributions (cited in his report which is mentioned in the bibliography appended to the present paper) Kappers has presented an interesting array of data to show that the definitive position of the perikarya of motor neurones in the brain of vertebrates is determined by "a process of taxis or tropism occurring under normal conditions of nervous action, that is, under the influence of reception and propagation of stimuli." This process he designates neurobiotaxis. It involves the conception of a bodily migration of the motor neurones towards the chief source of stimulation by the afferent neurones or through the tracts of the brain. A discussion of this conception as regards motor neurones does not belong in this paper on

the afferent system, but it is important to observe here that this paper describes specific instances of migration of cells masses in the form of several of the cranial ganglia. The clearest cases of this are the Gasserian and profundus ganglia, particularly the latter. This movement of the ganglia along the root towards the brain deserves more exhaustive and specific study, but some factors in the problem may be definitely stated. The ganglion of the ophthalmicus profundus V, for instance, in the earlier period is anchored to the skin far out over the eye. While this anchorage is intact root fibers make their connection with the brain. In embryos of the early flexure stage these fibers have established a firm anchorage to the brain by the bifurcation of the root beneath the external limiting membrane, and now their hold upon the skin is lost. Immediately a movement of the ganglion begins which finally carries it snugly up against the brain immediately around the entrance of the root (figs. 51 to 54). During this migration there are no apparent extrinsic mechanical factors introduced. A comparison of figures 1 and 2 shows that the migration has begun before the ganglion comes into contact with the eye, and comparison of figures 76 and 78 shows that after the ganglion comes in contact with the most dorsal portion of the eye it moves caudad over the surface of the eye—a median section through the eye in figure 78 showing only the tip of the ganglion while a similar section in figure 76 shows the massive portion of the ganglion. The ganglion moves caudad, therefore, with reference to the eye as well as with reference to the brain and auditory vesicle. There must be, then, some intrinsic factor involved in this movement.

As such a factor the root fibers with their firm anchorage within the brain immediately suggest themselves (fig. 22). As has been described above, these fibers are growing very rapidly caudad towards the chief motor centers in the lower portion of the medulla oblongata and the upper portion of the spinal cord. This growth may be regarded not simply as an increase in mass but as an active stretching out of the whole fiber in an amoeboid fashion after the manner of the movements of nerve cells growing *in vitro* as Harrison ('10, '11) has described them. In other

words, the entire axone may be thought of as creeping inward past its point of anchorage at the surface of the brain, as an amoeba would creep between obstacles, and as dragging the perikaryon after it. What the stimulus for such an action may be I shall not discuss here. I only wish to emphasize that this is a concrete case of migration of a group of neurones and that the rapid advance caudad of the root fibers within the brain and the concomitant shortening (not wrinkling) of the extracerebral root fibers convinces me that the axones are active factors in this process.

Another feature of the development of the sensory cranial nerves deserves mention in connection with the question of neuro-biotaxis, namely, the relative rates of development of the nerve trunks and their respective roots. In case of the trigeminal nerve it has been noted in the anatomical part of this paper that the root connections with the brain are well established at a time when no peripheral fibers can be traced to the skin. The lateral line primordia, also, are very small and are reached directly by the corresponding ganglia at a time when the latter have relatively long roots connecting with the brain. The most conspicuous case of this kind, however, is the visceral sensory system, the ganglia of which are connected with the placodes and are without peripheral nerves, excepting a few fibers of indefinite distribution from the geniculate ganglion, at a time when the root fibers have already formed a well defined fasciculus solitarius which reaches from the facial root to that of the vagus.

It is apparent, then, that in the development of the sensory cranial nerves the axone runs far ahead of the dendrite. This suggests the inference that, from the point of view of taxes or tropisms, normal peripheral excitation does not stimulate the growth of the axone inward. This is not meant to imply that there can be no nervous processes in these neurones before the dendritic terminals are established, but that, if there are such processes, they can have no reference to such stimuli as normally act upon the corresponding definitive nerves and have significance in the reactions of the animal. Similar conditions

prevail in the development of the reflex mechanism of the trunk, where the Rohon-Beard cells have their typical connection with the skin and the motor cells have their terminals established upon the muscles for a considerable time before they become incorporated into a functional reflex arc and are, therefore, of any value to the animal as regards behavior. From these facts it appears, then, that the behavior-value of nervous processes, if there be such in these nerve cells of early stages of development, has no regulating influence in establishing the primary plan of the integrating mechanism of the organism. What this regulating agency may be is one of the chief topics of interest in my study, but it can be best discussed upon the basis of an exhaustive anatomical and physiological analysis of the development of the various elements of the central nervous system during this early period. Such a study I already have well under way.

Since this paper was submitted for publication new evidence of a 'general chemical sense' in the skin of animals has been published by Crozier ('16) with particular reference to my suggestion ('14, pp. 205-207) that the stimulating effect of substances in solution may be accounted for by their destructive action upon the epithelial cells. He concludes that there is "no ground for Coghill's assumption that the cells of the germinative layer of the epithelium of fishes and amphibians are exposed to the action of the stimulating agent and thereby disrupted; and there is no histological evidence of disruption."

Without entering into further discussion or expression of opinion I wish here merely to raise the following questions, which seem to me to require settling before the main question at issue can be regarded as closed:

1. Since the skin of the invertebrate is very different anatomically from that of the vertebrate (the earthworm, for instance, having sensory nerve cells in the skin with endings free on the surface) may the reactions of the one be legitimately judged upon the basis of the structure and function of the other?

2. Since, according to Crozier the "concentrations of irritants employed by Parker and others" "do not penetrate at all" are there free nerve endings at the surface of the skin of vertebrates to function as receptors for these chemical stimuli (see Herrick-Coghill, '98)?

3. Since the epidermis of fishes and amphibians may be thought of as a system of three colloidal agglomerations, normally in equilibrium with each other—(1) the cellular protoplasm, and (2) surface films of mucus-like substance, which bathe opposite surfaces of (3) the cell membrane—may not very great disturbances of equilibrium with consequent violent effect upon neighboring parts result from the addition of a foreign solvent or solute to the surface film actually without the added solvent or solute penetrating the cell membrane (Nelson, '13, particularly table 5)?

4. In order to stimulate the tactile nerve endings mechanically through the reaction of the epithelial cells must the action of the reagent be so violent as to produce histologically or chemically demonstrable effect upon these cells? May not relatively slight increase or decrease in the turgor of the epithelial cells be sufficient to stimulate?

5. Why should the stimulating effect of water when applied after the application of a chemical stimulus be evidence of destruction of tissue in one case ("If any serious disintegration were produced by these solutions, it would be reasonable to expect the continuance of activity after external supply of the stimulant had been removed." Crozier, '16, p. 3) and in another case be evidence of a 'general chemical sense' ("Washing the foot with distilled water does not lead to cessation of the contraction, because, after exposure of the foot to certain solutions (Loeb '05), water stimulates" Crozier, '16, p. 5)?

6. How can this last mentioned irritability to water after chemical stimulation be explained upon the hypothesis of a "general chemical sense," and if it can be so explained why should it appear so conspicuously in the behavior of amphibian embryos (Coghill, '14, pp. 197, 198) the responses of which, Crozier con-

cedes, are probably "not at all due to sensory stimulation by acid?"

7. Granting that the so-called chemical stimuli act indirectly through the epithelial cells upon the nerve endings mechanically, may not the apparent physiological separation of the tactile and chemical receptors by use of cocaine still be accounted for upon the basis of the comparative strength of stimuli (Coghill, '14, pp. 206, 207)?

8. Regarding psychological differentiation of the two forms of stimuli as applied to the inner surface of the cheek of man, are the components of the cranial nerves of man sufficiently known to warrant the inference that morphologically equivalent receptors are concerned in the oral cavity of man and in the skin of the fish or frog? May not the visceral sensory system be a factor in the irritability in the oral cavity?

Some of these questions I have under investigation and I shall probably discuss them further in a later paper. Meantime, in the interest of the leading problem of my investigations, I shall hope to see Crozier's methods of inquiry projected further into the question concerning the existence of a 'general chemical sense' in the skin of vertebrates.

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EXPLANATION OF FIGURES

All of the figures are taken from *Amblystoma punctatum* Cope, excepting figure 71, which is from *A. microstomum* Cope, and figures 11 and 12, which are from another species of *Amblystoma*, probably *jeffersonianum*.

Methods. The methods followed in the preparation of Nos. 444, 449, 467 and 473 were described in Paper I. In Nos. 543, 546, 545, 550, 557 and 593 the fixation was with Van Gehuchten's fluid, and staining with erythrosin and toluidin blue. For No. 455 fixation was with formol-Zenker solution; staining with Bömer's haematoxylin and slightly acidulated orange G. For Nos. 561, 562 and 567 fixation was with Zenker's solution and staining with iron haematoxylin and orange G., excepting that the latter was omitted in No. 567. For 447 and 496 the fixation was with corrosive sublimate-acetic acid mixture and staining with alum cochineal (447) and alum carmine (496) *in toto* with Lyon's blue in 95 per cent alcohol on the slide. No. 635 was prepared with Paton's silver method: neutral 10 per cent formalin about three months [formalin (40 per cent formaldehyde) is saturated with sodium carbonate or lithium carbonate and the fixing fluid is made by mixing one part of this stock solution with nine parts of distilled water]; washed in running water over night; $\frac{3}{4}$ per cent silver nitrate five days and four hours; $\frac{3}{4}$ per cent nitrate of silver to which four drops of sodium hydroxide have been added for every 20 cc. of the solution, and then about twelve drops of ammonia, two hours; bath in distilled water to which one drop of glacial acetic acid has been added for every 2 cc., twenty minutes; 1 per cent freshly dissolved hydrochinone, sixteen hours; washed in distilled water; dehydrated through graded and absolute alcohol in about $6\frac{3}{4}$ hours; cleared and embedded through cedar oil, cedar oil one part and paraffin one part, cedar oil one part and paraffin two parts, then pure paraffin, in two hours or a little less. My experience is that this method of clearing and embedding is very satisfactory in use upon amphibian embryos, in which the yolk is a very refractory element. The silver-impregnated material does not suffer from treatment with cedar oil for a day or more.

ABBREVIATIONS

Ad., area of adhesion between the skin and ganglion or otocyst as indicated
Aud.V., the auditory vesicle or otocyst
c, the branch of the n. mandibularis V to the balancer
d, ramus palatinus VII
DC, the Rohon-Beard cells of the spinal cord and cells of like character in the brain
DT, the dorsal sensory tract of the spinal cord and its extension into the brain
e,n. hyomandibularis VII
Ec.Th., thickened regions of ectoderm
End., the endolymphatic appendage of the otocyst

Ent., the point of entrance of the nerve indicated into the brain
Fas.sol., the fasciculus solitarius
g, ramus lingualis of the glossopharyngeal nerve
G.G., the Gasserian ganglion
G.gen., the geniculate ganglion
G. Jug., the jugular ganglion of the vagus
G.L.L.VII, the lateral line ganglia of the facial nerve
G.L.L.IX, the lateral line ganglion of the glossopharyngeal nerve
G.L.L.X, the lateral line ganglion of the vagus

- G.oph.*, the ganglion of the ophthalmicus profundus
G.VIII, the ganglion of the auditory nerve
G.Vis.IX, the visceral ganglion of the glossopharyngeus
G.Vis.X, the visceral ganglion of the vagus
h, the postbranchial division of the first ramus branchialis vagi
L.o.p.V, the lateral terminal branch of the ophthalmicus profundus
L.L.VII, lateral line fibers of the facial nerve within the brain
L.L.VII,Asc., the ascending divisions of the lateral line root fibers of the facialis
L.L.VII,Des., the descending divisions of the lateral line root fibers of the facialis
L.L.IX,X, the lateral line root fibers of the glossopharyngeus and vagus within the brain
L.L.IX,X,Asc., the ascending divisions of the lateral line root fibers of the glossopharyngeus and vagus
L.L.IX,X,Des., the descending divisions of the lateral line root fibers of the vagus
M, myotome, the third in figures 1 to 4
Mdb.V, ramus mandibularis trigemini
Mes., mesenchyma
M.o.p.V, the mesial terminal branch of the ophthalmicus profundus
Mx., ramus maxillaris trigemini
Neu. II, neurones of the second order in the reflex circuits
Nuc.vis.m., the visceral motor nucleus
Olf., the olfactory organ
Op.St., the optic stalk
o.p.V., the ramus ophthalmicus profundus
P.L., the pigment layer of the retina
R.G.G., root of the Gasserian ganglion
R.G.Jug., root of the jugular ganglion, or the somatic sensory root of the vagus
R.L.L.VII, the lateral line root of the facialis
R.L.L.IX,X, the lateral line root of the glossopharyngeus and vagus
R.M., the root mass, a collection of chiefly indifferent cells about the entrance of the nerve roots into the brain
R.oph., the root of the ophthalmicus profundus
R.V., root of the trigeminus
R.V,m., the motor root of the trigeminus
R.VII,m., the motor root of the facialis
R.VII,vis., the visceral sensory root of the facialis
R.VIII, the root of the auditory nerve
R.Vis.IX, the visceral sensory root of the glossopharyngeus
R.Vis.X, the visceral sensory root of the vagus
R.X,m., the motor root of the vagus
R.XI, the spinal accessory root, or the most caudal motor root of the vagus
t, a dorsal branch of the ramus ophthalmicus profundus
Tr.Asc.V, the ascending trigeminal tract
Tr.Des.V, the descending trigeminal tract
Tr.S., a tract in the motor zone, probably bulbo-spinal
V, nervus trigeminus
VC, the somatic motor neurones of the ventral column
VII, nervus facialis
VIII, nervus acusticus
VT, the somatic motor tract
X, nervus vagus

Figs. 1 to 4 Graphic projections from serial, transverse sections upon the median plane to show the ganglia and sensory nerves of the head in the four physiological stages:

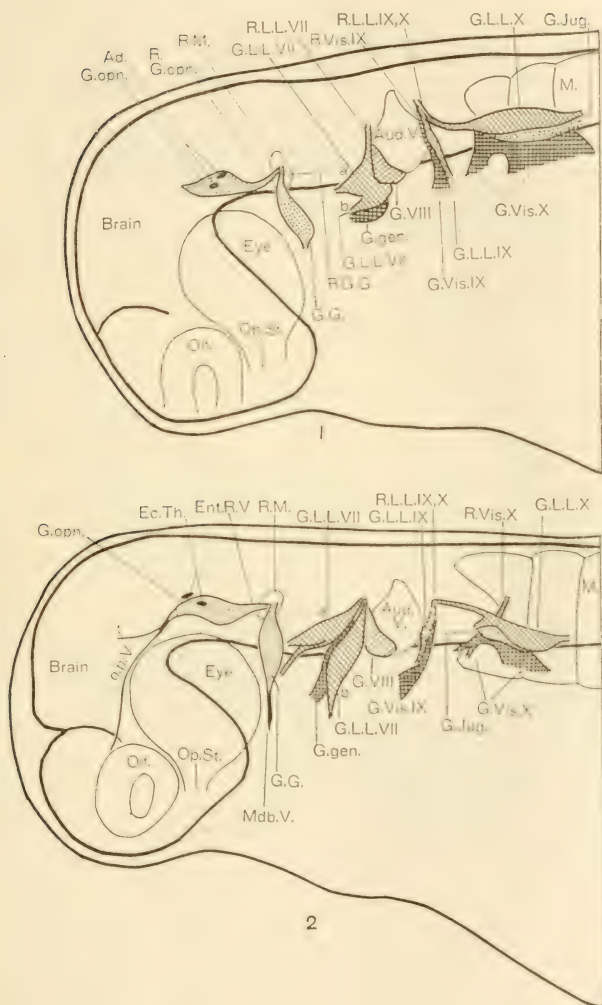
Fig. 1 (No. 467), the non-motile.

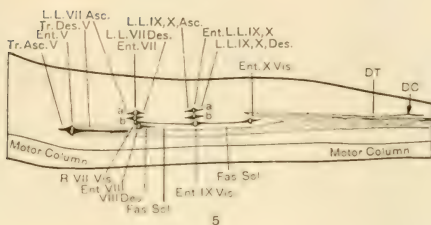
Fig. 2 (No. 473), the early flexure.

Fig. 3 (No. 449), the coiled-reaction.

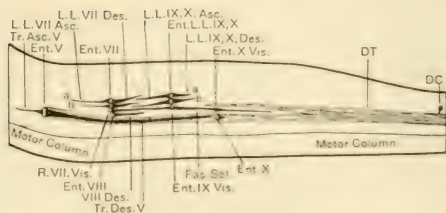
Fig. 4 (No. 444), the early swimming stage. $\times 50$.

The brain is drawn in heavy lines; the myotomes and sense organs, in light lines; the general somatic sensory ganglia, in stipple; the lateral line and auditory ganglia, in diagonal lines; the visceral sensory ganglia, in rectangular cross-hatching. The lateral line nerves are not fully represented here. They follow the course of the lateral line primordia, the distribution of which is shown in Paper I, figures 56 to 59. These figures with the descriptions in the text of this paper will give the important features of the lateral line nerves in the stages described. Silver impregnations of the early swimming stage show that these nerves are practically coextensive with the primordia of the organs. This is probably true of the earlier stages also, although satisfactory impregnations of the fibers have not yet been secured. The ganglia and sense organs have been projected mechanically with great care. The nerve trunks, which are too small to project distinctly at the magnification which has been used, have been sketched free-hand.





5



6

Figs. 5 and 6 Projections from serial, transverse sections upon the sagittal plane, of the sensory root fibers after they have entered the medulla oblongata, in the coiled-reaction stage (fig. 5, No. 449) and in the early swimming stage (fig. 6, No. 444). $\times 50$. The most rostrally located Rohon-Beard cell of the spinal cord is represented in each case, and the ascending fibers of these cells are shown as far cephalad as the root of the vagus nerve. The approximate extent of the trigeminal root fibers caudad is indicated in solid black, while the whole fiber system which I have called in the text the descending trigeminal tract but which is made up in large part of neurones of the second order is indicated in light lines about and beyond the root fibers proper. These figures are in a measure schematic, as will be seen by comparing them with figures 38 to 70, with which they should be studied.

Figs. 7, 8, 9 Non-motile stage (No. 467). $\times 50$.

Fig. 7 (section 1-3-12) illustrates the adhesion of the ophthalmic ganglion to the skin (*Ad.G.oph.*) dorsally of the eye, and the relation of the eye to the olfactory epithelium (*Olf.*).

Fig. 8 (section 1-4-3) is 120μ caudad of figure 7 and shows the constricted nature of the root of the ophthalmic ganglion, although at this level it is still ganglionic, also the relations of the retina, optic stalk and brain.

Fig. 9 (section 1-4-8) shows the adhesion of the Gasserian ganglion (*G.G.*) to the skin at *Ad.*, just ventrally of the lateral line primordium (*Sup.L.L.*). These drawings are made from the same embryo as is figure 1, with which they should be studied.

Fig. 10 Non-motile stage (No. 546, section 4-3-1). $\times 50$. The section lies approximately in the frontal plane, and shows favorably the two divisions of the ophthalmic ganglion (*G.oph.*) and the adhesion of one of these divisions with the skin (*Ad.*). Farther caudad the Gasserian ganglion appears in section (*G.G.*). A little farther caudad is the ectodermal thickening that is associated with the spiracular pouch (*Ec. Th.*).

Figs. 11 and 12 Non-motile stage (No. 455, sections 2-1-10 and 13). $\times 50$. The plane of section is longitudinal and latero-ventral, favorable to show the relations of the ophthalmic and Gasserian ganglia and their roots.

Fig. 11 shows the two divisions of the ophthalmic ganglion, *a* and *b*. Fig. 12 is 30μ farther ventrad and shows the root connections of these nerves with the brain (*R.V.*). Around the base of these roots is a mass of cells (*R.M.*) which does not seem to be a part of the ganglia at this stage. I have called this the root mass. The preauditory lateral line ganglion and the visceral sensory ganglion of the facial nerve (*G.VII*) are represented as a single mass. Its rostral end is in contact with the entoderm of the spiracular pouch.

Figs. 13, 14 and 15 Early flexure stage (No 473, sections 1-3-14, 1-4-6 and 1-4-12 respectively.) $\times 50$. These sections are selected from the series from which figure 2 was made, and should be compared with it.

Fig. 13 illustrates the ophthalmic ganglion after it has detached itself from the ectoderm, leaving an ectodermal thickening at *Ec.Th.*

Fig. 14 shows the attenuation of the root of the ophthalmic ganglion (*R.oph.*) 160μ caudad of the last figure and at the level of the caudal portion of the eye.

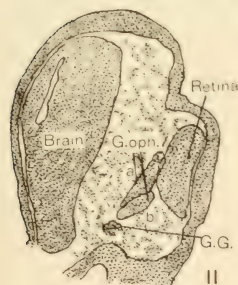
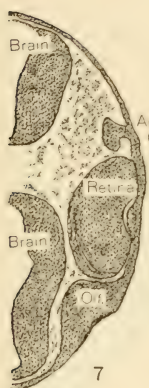


Fig. 15 is $60\ \mu$ still farther caudad, showing the Gasserian ganglion (*G.G.*) connected by a slender root with the root mass (*R.M.*) at the surface of the brain, and the relation of this ganglion to the lateral line primordium (*Sup.L.L.*).

Fig. 16 Early flexure stage (No. 550, section 1-2-14). $\times 50$. This figure is introduced to show the plane of section of figure 22, which is taken from the same section.

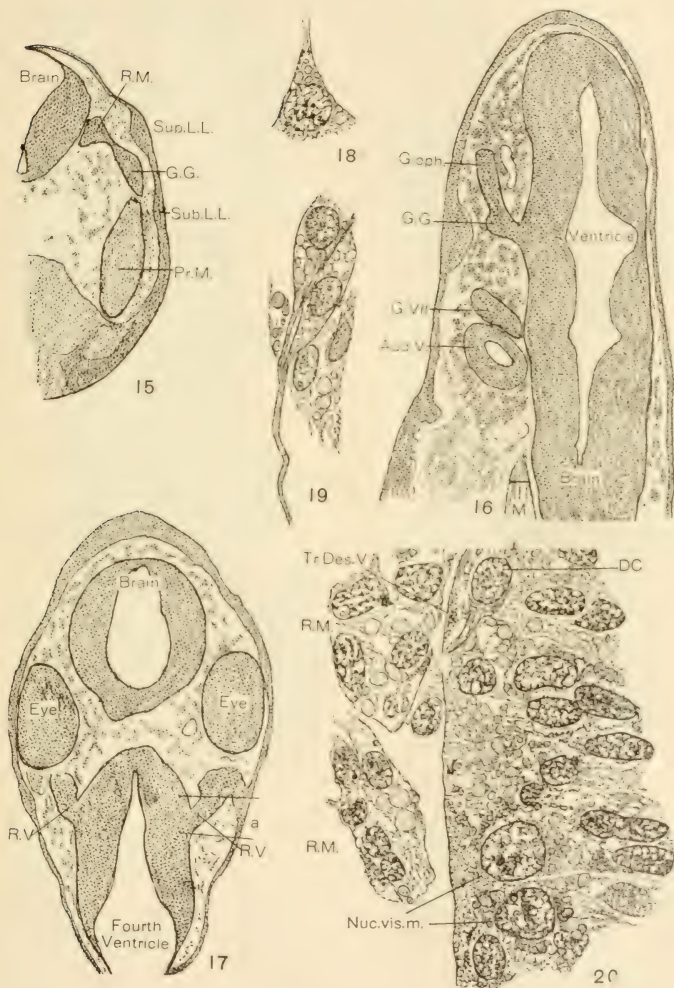
Fig. 17 Coiled-reaction stage (No. 561, section 1-3-14). $\times 50$. This figure is introduced to show the plane of section of figure 33, which was taken from the region indicated between the lines at *a*.

Fig. 18 Early flexure stage (No. 545, section 5-2-14). $\times 500$. A ganglion cell with its peripheral, dendritic process, in the distal portion of the ophthalmic ganglion. The spherules in the cytoplasm are yolk.

Fig. 19 Early flexure stage (No. 593, section 2-5-7). $\times 500$. Ganglion cells of the Gasserian ganglion, with axones reaching towards the brain. Other indifferent cells appear around the ganglion cells. The yolk is indicated in the cytoplasm as in the last figure.

Figs. 20 and 21 Non-motile stage transverse section (No. 543, sections 3-3-16 and 13, respectively). $\times 500$.

Fig. 20 is $15\ \mu$ caudad of figure 21, and shows root fibers of the trigeminal nerve within the brain (*Tr.Des.V*). Both figures show cells of the root mass (*R.M.*) Fig. 21 a large cell (*D.C.*) which has the anatomical features of Rohon-Beard cells of the spinal cord sends a process out of the brain into the trigeminal root. A similar cell (*D.C.*) appears in figure 20, in which figure the motor nucleus of the trigeminus (*Nuc. vis.m.*) is seen to be in a much more ventral position.



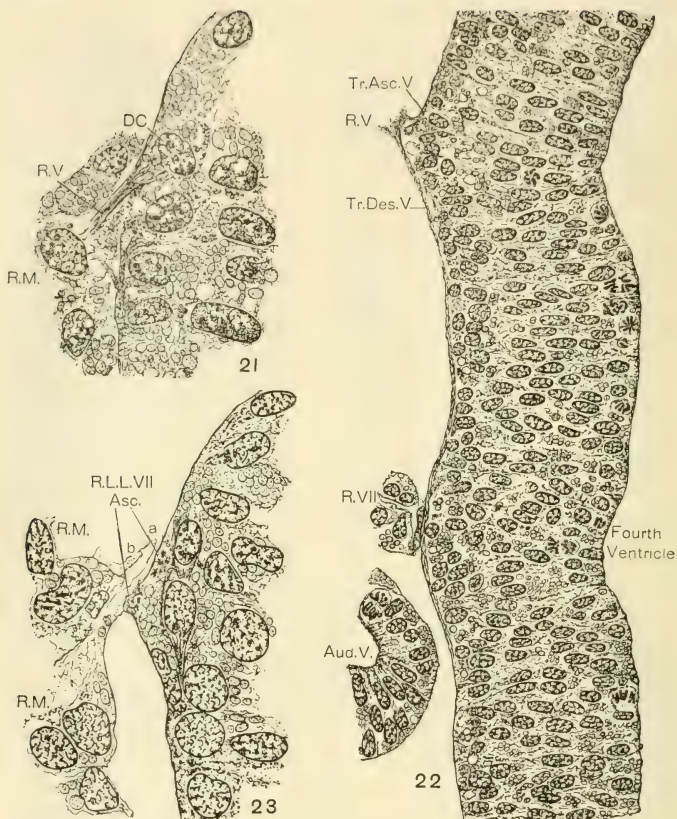
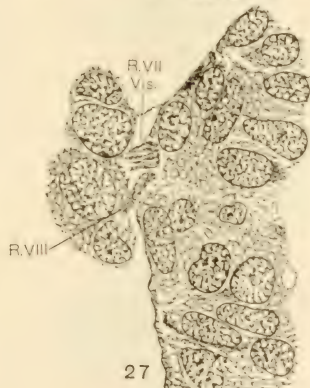
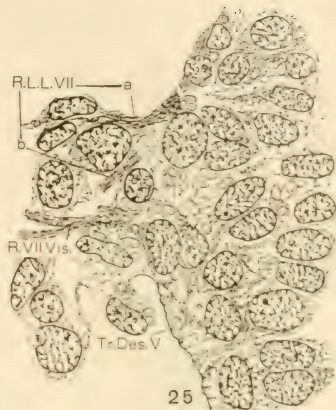
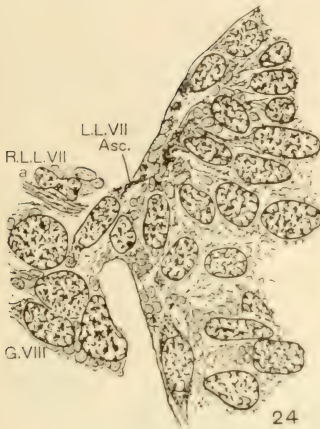


Fig. 22 Early flexure stage (No. 550, section 1-2-14). $\times 200$ The plane of section of this figure is illustrated in figure 16. It illustrates a longitudinal section of the medulla oblongata, in which the entrance of the trigeminal root appears (*R.V.*). There is a short ascending trigeminal tract (*Tr.asc.V*) and a descending trigeminal tract (*Tr.des.V*) which reaches to the level of the middle of the auditory vesicle (*Aud.V.*) The ventricular pits opposite the entrance of the roots are conspicuous features of such a section.

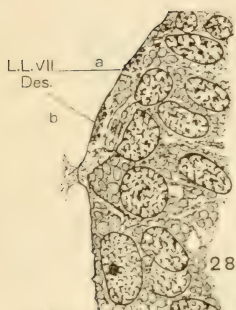
Figs. 23 to 28 Early flexure state (No. 473, sections 1-5-14 to 19). $\times 500$. These figures are from six successive sections of the series from which figure 2 was made, and show the relations of the root bundles of the seventh and eighth



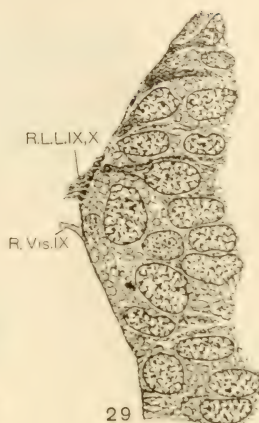
nerves as they enter the brain. Figures 25 and 26 show the root fibers of the lateral line component (*R.L.L.VII*, *a* and *b*) and figures 24 and 23 show the ascending divisions of these root fibers (*L.L.VII, Asc.*). Figure 25 shows also the root fibers of the visceral sensory component (*R.VII.vis.*) of the facial nerve approaching the brain amid cells of the root mass, and figure 27 shows this component entering the brain. Immediately ventrally of this is the root of the eighth nerve. In the next section caudad, figure 28, descending divisions of the lateral line root fibers (*L.L.VII, Des.*) appear, but nothing can be seen of the other components of this complex. The entire cephalocaudal range through which the lateral line component can be recognized in this series within the brain is only 60μ .

Figs. 29 to 31 Early flexure stage (No. 473, sections 1-6-16 to 18). $\times 500$. three successive transverse sections through the entrance of the root of the glossopharyngeal nerve into the brain. The lateral line component (*R.L.L.IX,X*) enters in figures 29 and 30, and the visceral sensory component (*R.Vis.IX*) enters in figures 30 and 31 at a slightly more ventral and caudal level than the entrance of the lateral line component. No ascending or descending fibers of this complex can be made out beyond the limits of these three sections, a range of $30\ \mu$.

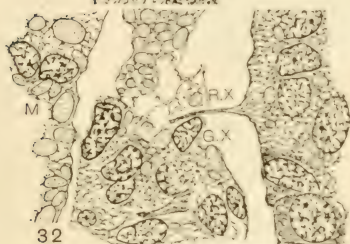
Fig. 32 Early flexure stage (No. 473, section 2-1-12). $\times 500$. This figure shows the only perceptible connection of the vagus nerve other than the lateral line root with the brain at this time, a single nerve fiber (*R.X.*), which is of doubtful nature. It may be motor.



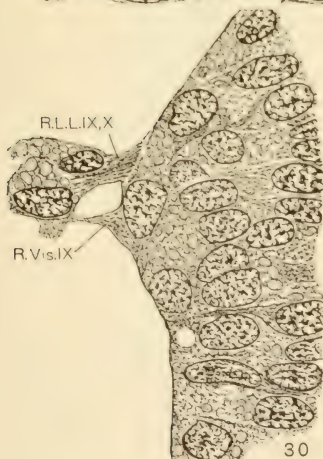
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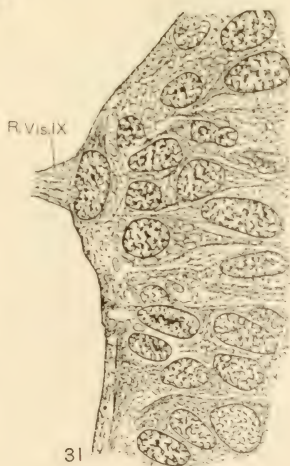
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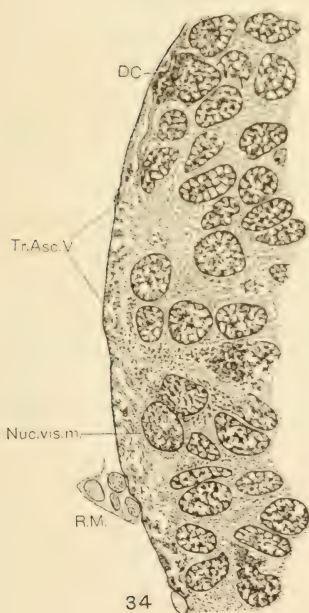
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Fig. 33 Coiled-reaction stage (No. 561, section 1-3-14). $\times 500$. The plane of section is shown in figure 17, in which the region of this figure is included between the lines and indicated by *a*. The caudal end of the figure is to the left, and is a great deal farther dorsad than is the rostral or right end of the figure. The root of the trigeminal nerve (*R.V*) here approaches the brain in two divisions, the root from the Gasserian ganglion (*R.G.G.*) and that of the ophthalmic ganglion (*R.oph.*). The latter is entering the brain and bifurcating into a short ascending tract (*Tr.Asc.V*) and a massive descending tract (*Tr.Des.V*). In the angle of this bifurcation is a large neurone (*D.C.*), the very large nucleus of which is in sharp contrast with the surrounding nuclei. This cell lies in the dorso-ventral level of the sensory root and is far removed from the motor nucleus, several cells of which can be recognized in a more ventral position (*Nuc.vis.m.*).

Fig. 34 Coiled-reaction stage (No. 562, section 1-4-2). $\times 500$. This is from a transverse section located 56μ rostral of the entrance of the trigeminal root. It shows the presence at this level of ascending root fibers of this nerve (*Tr.Asc.V*). In a more dorsal position there is a cell of the Rohon-Beard type (*D.C.*) which has a long process extending into the immediate vicinity of or actually into the ascending trigeminal tract.

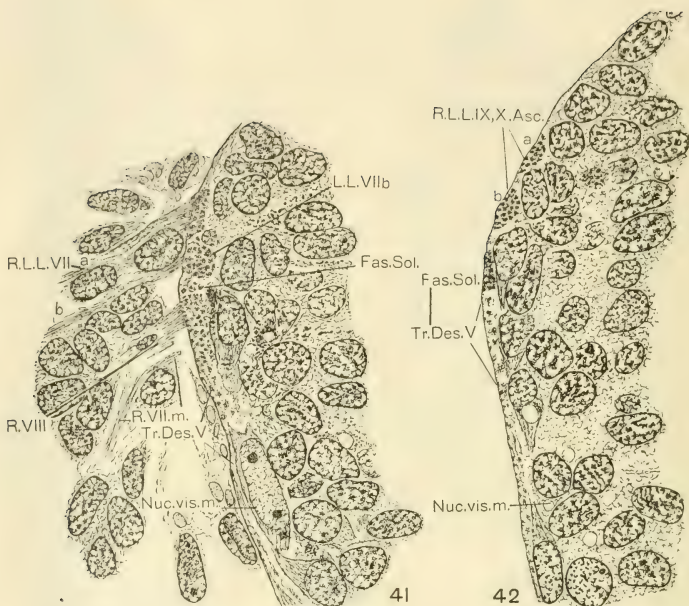
Figs. 35 to 37 Coiled-reaction stage (No. 562, sections 1-4-9, 10 and 11). $\times 500$. Three successive transverse sections through the brain at the entrance of the trigeminal root (*R.V*).

Figure 35 is the most rostral in position, and shows two or three cells of the type described in the last two figures (*D.C.*). Another cell of this type appears in figures 36 and 37 (*D.C.*), two sections through the same cell. In both figures the peripheral process of the cell is shown entering the trigeminal root. In figure 37 there is a stump of a dorsally directed process, and in figure 36, evidence of a ventrally directed process. Cells of the root mass (*R.M.*) appear in all these figures.



Figs. 38 to 41 Coiled-reaction stage (No. 447, sections 1-5-10 to 13). $\times 500$. Four successive, serial, transverse sections through the entrance of the seventh and eighth nerves into the brain. These figures are from the same specimen as are figures 3 and 5, with which they should be studied. Figure 38 is the most rostral of the series. In this figure the root of the lateral line ganglion on the hyomandibular division of the nerve (*R.L.L.VII,b*) is entering, and the ascending fibers of the other division (*L.L.VII,Asc.a*) appear in a slightly more dorsal position. These fibers are seen entering the brain in the three following figures (*R.L.L.VII,a*). The visceral sensory component of the facial nerve is entering the brain in figure 39, and its fibers appear as the fasciculus solitarius (*Fas.Sol.*) in the two following figures. The auditory root (*R.VIII*) enters in figures 40 and 41. All of these root systems lie dorsally of the descending trigeminal tract (*Tr.Des.V*), which consists here of scattered fibers through a rather extensive zone.



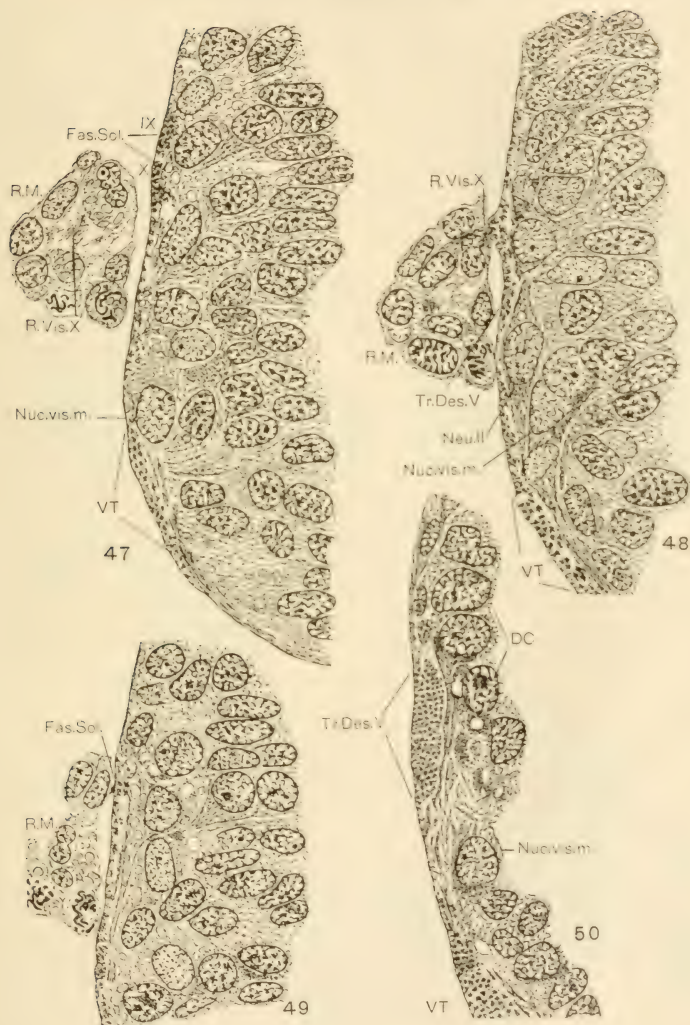


Figs. 42 to 46 Coiled-reaction stage (No. 447, sections 2-1-6 to 10) $\times 500$. Five successive serial, transverse sections through the entrance of the glossopharyngeal nerve into the brain. Figure 42 is the most rostral. In it can be readily recognized the ascending fibers of the lateral line component of the ninth and tenth nerves (*L.L.IX,X,Asc.a* and *b*), and ventrally of these the visceral sensory component of the facial nerve as the fasciculus solitarius (*Fas.Sol.*). The lateral line components are entering the brain in the three successive figures, while the visceral sensory (*R.Vis.X*) enters in figure 44. In the two following figures this component appears with the facial fibers as the fasciculus solitarius, and in figure 46 the descending divisions of the lateral line roots occur (*L.L.IX,X,Des.a,b*). The descending trigeminal tract (*Tr.Des.V*) appears as scattered fibers immediately beneath the external limiting membrane throughout this region. Neurons of the second order (*Neu. II*) stretch across the whole mesial face of this system of root fibers.



Figs. 47, 48, 49 Coiled-reaction stage (No 449, sections 1-6-17, 18, 19). $\times 500$ Three successive serial transverse sections through the brain at the level of the entrance of the root of the vagus nerve. Figure 47 is the most rostral of this set. In it the visceral sensory root of the vagus (*R.Vis.X*) is approaching the brain surrounded by cells of the root mass (*R.M.*), and the fasciculus solitarius appears in two clusters of fibers (*Fas.Sol.*), a more dorsal representing the facial and glossopharyngeal portion and a ventral consisting of ascending fibers of the vagus root. This figure, as well as the following, extends ventrad to show the relation of the sensory centers to the motor column (*VT*), and the visceral motor nucleus (*Nuc.vis.m.*). The root fibers of the vagus enter in figure 48, and in the following figure a few descending root fibers can be recognized (*Fas.Sol.*).

Fig. 50 Early swimming stage (No. 567, section 2-1-19). $\times 500$. From a transverse section through the medulla oblongata 35μ caudad of the entrance of the trigeminal nerve. Around the dorsal margin of the descending trigeminal tract (*Tr.Des.V*) is a cluster of three or more large neurones (*DG*) of the giant ganglion cell type. Their processes run ventrad towards the motor nucleus of the trigeminus (*Nuc.vis.m.*).



Figs. 51 to 54 Early swimming stage (No. 557, sections 2-1-1,5,7,11). $\times 200$. Four sections selected from a transverse series through the brain in the vicinity of the entrance of the root of the trigeminal nerve. The massing of the ganglia against the brain is shown here, a relation which should be noted in the study of figures 1-4. Figure 51 is the most rostral in this set, and figure 54 the most caudal. In figure 51 the ophthalmic ganglion (*G.oph.*) appears with a crescentic piece of the Gasserian ganglion (*G.G.*) on its ventral surface. The fibers from this ganglion enter in figure 52 (*R.oph.*), while the root of the Gasserian ganglion enters in figure 53 (*R.G.G.*) more dorsally. This section is 28μ caudad of the last. In figure 54 the motor root of the nerve appears.



Figs. 55 to 60 Early swimming stage (No. 496, sections 1-5-4 to 9). $\times 500$. Six successive serial transverse sections through the entrance of the roots of the seventh and eighth nerves into the brain. Figure 55 is the most rostral of the set. In this figure the ascending divisions of the lateral line component (*L.L. VII, Asc. a, b*) and the roots of the visceral sensory component (*R. VII, Vis.*) and the auditory root (*R. VIII*) are beginning to enter. The lateral line roots (*R. L. L. VII, a, b*) enter in figures 56 to 59. In figure 58, 59 and 60 the fibers of the visceral sensory component have become the fasciculus solitarius (*Fas. Sol.*), and the descending auditory root fibers (*VIII, Des.*) appear along the dorsal margin of the descending trigeminal tract (*Tr. Des. V*). Neurones of the second order (*Neu. II*) here lie across the entire sensory zone. These figures should be compared with figure 6.

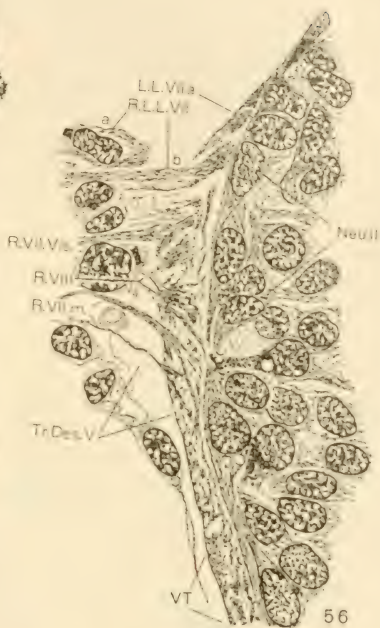
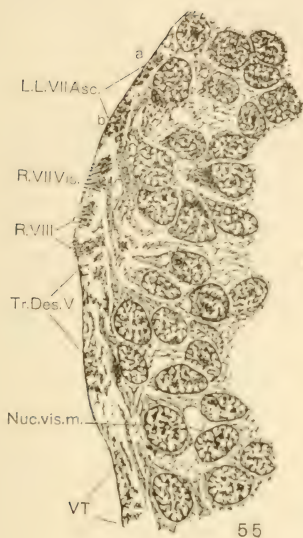
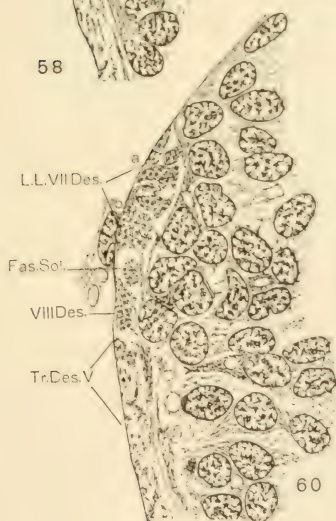
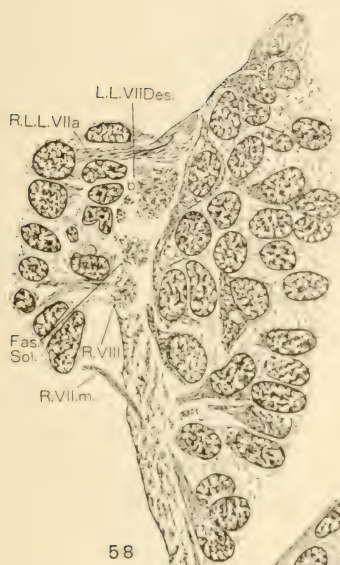


Fig. 61 Early swimming stage (No. 496, section 1-5-15). $\times 500$. From the same series as figures 55 to 60, at the level of the middle of the auditory vesicle and endolymphatic appendage (*End*). This should be compared with figure 6. It shows the relations of the descending facial and ascending postauditory lateral line components in this early stage; also the fasciculus solitarius (*Fas.Sol.*) and the descending auditory fibers (*VIII Des.*). Mesenchymal cells are now pressing in between the brain and the otocyst.



Figs. 62 to 65. Early swimming stage (No. 496, sections 1-5-24 to 1-6-2). $\times 500$. Four successive transverse serial sections through the entrance of the glossopharyngeal nerve into the brain. Figure 62 is the most rostral. The lateral line roots (*R.L.L.IX,X*) enter in figures 63 to 65, and on their ventral surface the visceral sensory root (*R.Vis.IX*) enters in figures 63 and 64. These figures should be compared with figure 6.



Figs. 66 to 70 Early swimming stage (No. 496, sections 1-6-19 to 23). $\times 500$. Five successive serial transverse sections through the entrance of the root of the vagus nerve into the brain. Figure 66 is the most rostral in position. In this figure the fasciculus solitarius (*Fas. Sol.*) can be recognized between the descending trigeminal tract (*Tr.Des.V*) ventrally and the ascending fibers of the spinal Rohon-Beard cells dorsally (*DT*). The visceral sensory root of the nerve (*R.Vis.X*) enters in figures 67 and 68 and the somatic sensory root (*R.G.Jug.*) enters in figure 69; the visceral sensory root entering more dorsally. Figure 70 shows fasciculus solitarius fibers caudally of the entrance in a relation like that in figure 66, which is on the rostral side of the root. The somatic motor column is shown in 67, 69 and 70 (*VT*). The visceral motor nucleus appears in all the figures of this set, and lying between it and the external limiting membrane is a tract (*Tr.S*) which can not be described in detail in this paper. It is probably a bulbo-spinal tract. The figures of this set should be compared with figure 6.



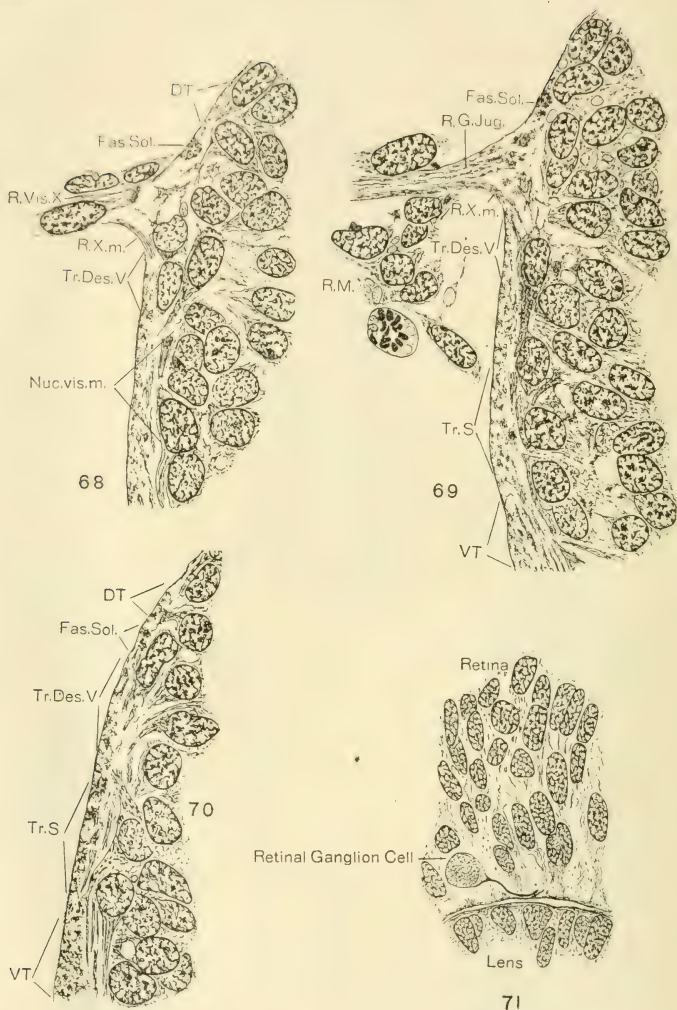


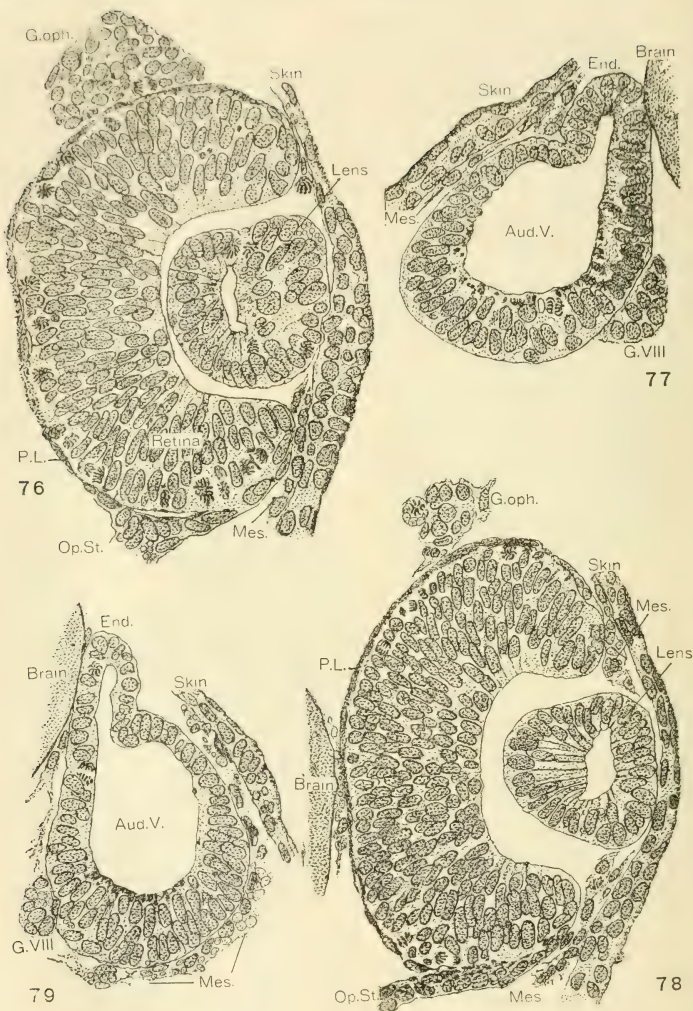
Fig. 71 Early swimming stage of *Amblystoma microstomum* Cope (No. 635, section 1-3-7). $\times 500$. A transverse section of the embryo through the eye, to show the nature of the ganglion cells of the retina and their fibers, which at this time decussate in the most rostral portion of the postoptic commissure. Silver impregnation after fixation in neutral formalin.



Figs. 72 to 79 From transverse sections through the eye and ear of the four stages.

Figs. 72, 73 non-motile (No. 467).

Figs. 74, 75 early flexure (No. 473).



Figs. 76, 77 coiled-reaction (No. 449); early swimming, figures 78, 79 No. 444). The sections through the eye were selected at levels which show the most extensive area of the lens, and those of the ear were selected at levels which show the clearest differentiation of the endolymphatic appendage.

THE EFFECT OF ACTIVITY ON THE HISTOLOGICAL STRUCTURE OF NERVE CELLS

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INTRODUCTION

The study of the effect of functional activity on the morphology of nerve cells has been the subject of numerous investigations in the past. These researches have covered a considerable range as to kinds of cells studied, degrees of fatigue, and as to methods of treatment of the material. There is almost complete agreement on one point, namely, fatigue results in appreciable changes in cell structure. There is, however, the utmost divergence of opinion as to the nature of the changes, and one who tries to correlate the findings of the different workers in this field is utterly confused. The chief findings relate to (a) size of cell body and nucleus and (b) amount and distribution of chromatic material. While one group of workers concludes that fatigue results in decrease in the size of cell and nucleus (Hodge (1), Van Durme (2), Luxemburg (3), Legendre and Piéron (4), Lugaro (5), and Nissl (6)), another group finds an increase in the size or a change in the nucleus-plasma relation (Crile and Dolley (7), Mann (8), Vas (9)). Lambert (10) noted no change in the size of cell or nucleus. Increase in chromatic substance (hyperchromatism) as the chief result of fatigue has been noted by Nissl. Most workers (Van Durme (2), Vas (9), Lugaro (5), Mann (8)) have described a decrease in chromatic material as

¹ These experiments were carried out at the suggestion and under the direction of Prof. Adolf Meyer, and completed in 1912. The computations were in part made after leaving Baltimore. It gives me great pleasure to express here my thanks and appreciation to Doctor Meyer for his keen interest in the experiments and for many helpful suggestions.

the result of exercise. Luxemburg (3) described a breaking up of the chromatic bodies into granules, and Dolley tried to reconcile these contradictions by regarding the hyperchromatism as a result of moderate activity, and the hypochromatism the result of excessive activity or exhaustion. They find that both stages may be present in the same animal simultaneously. Other changes, such as wandering of the nucleus toward the periphery of the cell (Magini (11), Lambert (10), Vas (9)), rupture of the nuclear and cell membranes (karyolysis and karyorhexis) have been described. Dolley noted thirteen stages of cell change from hyperchromatism to disintegration or death of the cell, corresponding to the degrees of moderate activity up to complete exhaustion. Eve (12), in a study of sympathetic nerve cells, concludes that "there are usually some small differences before and after stimulation, but these are nearly all inconstant and generally reversible. Such divergence in the results is not so surprising when we stop to consider the complicating factors necessarily attending these experiments, such as, (1) difficulty of separating the effects of normal activity from unavoidable shock or injury to the nervous system in killing the animal (the nervous system 'does not 'die' as soon as the heart stops beating); (2) postmortem changes ensuing between the time of death and complete penetration of the tissue by the fixing agent due to the action of autolytic enzymes present in all tissue; (3) varying chemical action of fixing agents; for example, formaldehyde coagulates protein by combination with the amino groups, alcohol by dehydration, sublimate by formation of salts, etc.; (4) the solvent action of materials used in fixation and in imbedding, for example, alcohol, xylol, paraffine; (5) varying effects of chemical reaction between basic or acid dyes used in staining and the different cell structures; (6) effect of subjecting tissue to temperatures of 50° to 54° in the paraffine oven for a period of several hours.

In the present series of experiments I attempted as much as possible to minimize the formation of artefacts, having in mind the above mentioned considerations. It was hoped that by using special care in the handling of material, the use of im-

proved technic in fixation and staining, and by varying the kind of degree of activity in a long series of experiments, using different animals and studying various kinds of nerve cells, that some degree of uniformity of results might be obtained.

METHODS

A resting control animal was used in each experiment. The animals were killed in most cases by bleeding after ether anaesthesia, the nerve material removed as quickly as possible, cut into small pieces, and the control and fatigue specimens placed in the same fixing solution, imbedded side by side in the same block of paraffine, cut with the same stroke of the knife, mounted and stained together on the same slide. The detailed data of experiments showing animals used, kind of stimulation, length of time, microscopic technic, etc., are shown in table 1. In all, fifteen experiments were performed. Examination of the table reveals the fact that almost every form of activity was used; normal activity, forced activity, activity resulting from electrical stimulation, both faradic and galvanic, chemical stimulation, and shock being applied in the experiments. The kinds of animals used were dogs, cats, rats, sparrows, pigeons, and frogs. The microscopic technic used was varied considerably, not only as to fixative but also as to staining fluid. The stain most frequently used was Held's modification of Nissl's method.

The examination of the material for comparison was facilitated by having both control and fatigue specimens mounted on the same slide. Changes such as have been previously described as resulting from fatigue were carefully examined for: namely, comparative amounts and distribution of chromatic substance, size of granules, nucleus-plasma relation, relative size of cells and nuclei, etc. In order to determine whether any change in the size of the cell had resulted from activity, a large series of camera lucida drawings were made. These drawings were made of cells without selection. A field was taken, and every cell showing a nucleolus was included. This precaution was necessary in order to be assured that the cell was cut through a com-

TABLE 1

FORM OF STIMULATION		ANIMAL	DURATION OF ACTIVITY	TISSUE STUDIED	FIXATION	STAIN
(A) Normal activity	walking and running	dogs	3½ hrs.	a. lumbar and cervical enlargement of cord b. cerebellum c. cruciate gyrus	Held's fluid and 95 per cent alcohol	Methylene blue and erythrosine after Held's modification of Nissl
	flying	sparrows	ca. 12 hrs.	a. brachial ganglia b. brachial enlargement c. whole brain	Held's fluid and 95 per cent alcohol	a. Held's b. Toluidine blue
		pigeons	ca. 12 hrs.	a. brachial enlargement b. brachial ganglia c. whole brain	Held's fluid and 95 per cent alcohol	a. Held's b. Toluidine blue
(B) Forced activity	swimming and running in a wheel	rats	ca. 12 hrs.	a. cervical enlargement b. lumbar enlargement c. brain	Held's fluid and 95 per cent alcohol	a. Held's b. Toluidine blue
	running in treadmill	dogs	1, 2½ and 5 hrs.	a. dorsal, cervical and lumbar ganglia b. cervical and lumbar ganglia c. cerebellum	Zenker's fluid Held's fluid	Polychrome methylene blue Held's method

Electrical stimulation	galvanic stimulation of leg	pigeon	4½ hrs. (stim. per sec.)	a. lumbar enlargement of spinal cord b. lumbar and dorsal ganglia	Alcohol	Toluidine blue Toluidine blue and eosin
	Faradic stimulation of sciatic nerve	cats	5 hrs. (15 sec. on and 45 sec. off)	a. sciatic ganglia b. lumbar cord	Formalin and alcohol	Held's stain polychrome methylene blue
		frogs	4½ hrs. 6 hrs.	a. lumbar enlargement b. lumbar ganglia	Held's fluid	Held's modification of Nissl
Drug stimulation	strychnine $\frac{1}{6}$ – $\frac{1}{3}$ gr. with perfusion of salt solution—O ₂	frogs	1–10 hrs.	cervical and lumbar cord	Held's fluid alcohol and formalin	Held's modification of Nissl polychrome methylene blue
	anaemia, 200 cc. blood removed, 4 kg. dog	dogs	5 hrs. comatose	cruciate gyrus and cerebellar cortex	10 per cent formalin	polychrome methylene blue

parable plane. Cells were first drawn using a low power for orientation, each cell numbered on the paper, then the one-sixth objective or the one-twelfth objective was used to project the cell, care being taken to draw always at the same distance from the microscope. The area of the cells was then computed with the use of the polar planimeter. The data are tabulated in table 2, and will be referred to later in connection with the individual experiments.

Normal Activity

Experiment I. Dogs. The animals which served for this experiment were two fox terrier puppies from the same litter, three months old. A female was used for activity while the male served for the control. The latter remained quiet in a cage, where he had been kept for several weeks previously. The activity animal was led by a chain on a fast walk into the country; the distance covered was fifteen miles in three and a half hours. This was a considerable feat for a puppy of this size, as the pace meant running all the way for her. At the end of three and a half hours, she was so fatigued that she refused to go any further, and had to be carried home. She was then killed less than one hour after exercise had ceased, the brain and cord at once removed, and sections taken from the lumbar and cervical enlargements, from the cerebellum, and from the cruciate gyrus, and the sections placed in 10 per cent formalin and in Held's fluid. The control dog was killed at the same time, in the same way, and corresponding sections taken from the brain and cord, and placed in the same fixing fluid with those from the 'fatigue' animal.

MICROSCOPIC STUDY OF THE NERVE CELLS

Cervical enlargement of the cord

The cells are uniformly stained, the Nissl bodies standing out clear and distinct. In both control and fatigue specimens, there is an occasional cell showing slightly clear areas about the nucleus, but this is no more marked in either section, and these cells are as numerous in the control as in the other. Drawings were made with the Leitz camera lucida, using the one-twelfth objective and no. 2 ocular. The camera lucida outlines of the cells and nuclei were traced with a planimeter with the following result:

Control cells, 43 measured, average area 0.441 square inches.

TABLE 2
Volume expressed in square inches (the mean of each set recorded)

KIND OF NERVE CELLS	EXPERIMENT 1 DOGS		EXPERIMENT 2 SPARROWS		EXPERIMENT 3 PIGEONS		EXPERIMENT 4 RATS		EXPERIMENT 5 DOGS				EXPERIMENT 6 PIGEONS		EXPERIMENT 15 DOGS	
	R	F	R	F	R	F	R	F	R	F	F 1 hr.	F 2 1/2 hrs.	R	F	R	F
Anterior horn cells of cervical enlargement of cord.....	0.44	0.420			0.355	0.288			1.22	1.077	0.943	1.102				
No. of cells measured.....	43	36			8	9			14	10	9	8				
Anterior horn cells of lumbar enlargement of cord.....	0.563	0.754							0.1							
No. of cells measured.....	26	28														
Purkinje cells of cerebellum.....	0.21	0.22	0.182	0.174			{ 0.188 0.236 0.195 0.213 }		0.363	0.31	0.341	0.312			0.0753	0.097
Number of cells measured	42	25	30	30			{ 34 39 33 35 }		29	29	30	17			48	50
Ganglion cells of brachial ganglion.....			{ 0.199 0.190 * { 0.157 0.158 }						0.994	0.691	0.858	0.992				
Number of cells measured			54	54	47	48			50	50	54	53				
Posterior ganglion cells of sciatic nerve.....			48	44												
Number of cells measured									0.886	0.796	0.927	0.892	0.0515	0.0582		
										50	47	49	17	20		

* These represent separate counts made on different slides. Since the sections were not cut through identically the same region and might be of different thickness or fixed differently, it is considered more accurate to compare only those measurements made from the fatigue control sections which have been mounted on the same slide.

Fatigue cells, 36 measured, average area 0.420 square inches.

These results are tabulated along with the measurements of cells from other regions and experiments in table 2.

LUMBAR ENLARGEMENT OF THE CORD

Examination of sections stained according to Nissl's original method, e.g. "seifen methylen blue," after fixation with 95 per cent alcohol, and according to Held's modification of this stain. As regards amount and distribution of chromatic material, there is no difference between the control and fatigue sections. Several sections stained by Held's method showed grouping of cells near the dorsal part of the anterior horns where the chromatolysis was slightly more marked in the fatigue specimen than in the control. This paucity of chromatic substance was not only around the nucleus but also extended to the dendritic trunks. This was not constant in all sections nor throughout the same section. Cells in the extreme anterior horn show no difference from the control. It seems probable that the particular section cut through a nucleus of the cord where the character of the cells was slightly different, since the similar variation in favor of the control cells was observed in some of the subsequent experiments. The nuclei of the control and fatigue animals show no difference whatever in the amount and nature of the staining of the chromatic material.

CRUCIATE GYRUS

These cells are uniformly stained, the nuclei and nuclear membranes and chromatic bodies being distinct. No difference in the morphology of these cells can be made out with the highest power of the microscope.

CEREBELLUM

The cells of the cerebellar cortex stained with Held's method are well defined, and show a distinct architecture. Numerous cells were examined, but without any discoverable difference in the staining reaction between control and fatigue specimens.

Sparrows

Experiment 2. A male sparrow was shot and instantly killed with a rifle ball at 6 a.m. The brain, cord, and brachial and dorsal ganglia were removed one-half hour later. These were placed in Held's fluid and in 10 per cent formalin.

Fatigue bird. A male sparrow that had been flying about all day was shot, and the brain, cord, brachial and dorsal ganglia removed one-half hour later, and placed in Held's fluid and 10 per cent formalin.

Microscopic study. Brachial ganglia—one-twelfth objective, no. 2 ocular, Leitz. The cell architecture is distinct in both the fatigue and control specimens. Sections stained with Held's method show the cell bodies stained diffusely pink with distinct Nissl granules stained dark blue. There is no difference as regards depth of stain of either cell bodies or the amount and size or depth in staining of the Nissl bodies. No cells in either section showed any crenation of the nuclear membrane such as described by Hodge. Some cells show excentric nuclei and nucleoli, but by actual count this occurrence is just as common in the control specimen. Examination of the anterior horn cells of the spinal cord in the region of the cervical enlargement as well as the examination of the Purkinje cells of the cerebellum show no variation from the control morning specimens with respect to size of cells and nucleus or in the morphological markings.

Pigeons

Experiment 3. For this experiment pigeons were selected which were in the daily habit of making long flights, sometimes remaining on the wing for over an hour at a time. One of these pigeons was killed just at dusk, and as a control, one approximately of the same age was killed from the same flock at six o'clock the following morning. For study the entire brain was removed as well as the brachial ganglia and sections from the spinal cord in the region of the brachial and lumbar enlargements.

Microscopic study. Brachial ganglia. The evening (fatigue cells) show no crenation, no central chromatolysis, and there is no apparent difference in the size or distribution of the granules from the controls. Measurement of a large number of cells show that the differences in size of the cell bodies or nuclei in the fatigue and control specimens fall within the limit of "variation."

Anterior horn cells. Brachial cord. No difference in any respect could be detected between control and fatigue specimens.

Cerebellum. A large number of the cells were studied from both the evening and morning pigeons, but no constant variation in morphological markings could be detected.

Rats

Experiment 4. This and the following experiments differ from the preceding in that here the activity was forced to the point of exhaustion. In the previous experiments exercise was voluntary. Two half grown white rats from the same litter served for this experiment. They had previously been kept in a cage and well fed. The fatigue rat was kept running in a revolving wheel for one-half hour, having become tired, he refused to run, and clung to the wall of the wheel. The exercise was then changed from running to swimming. The rat was placed in a tank of lukewarm water, where he kept up constant swimming in an attempt to escape. At the end of one hour he was quite exhausted, was taken out, and allowed to rest for an hour. He was then made to swim a half hour again, followed by a half hour of rest. This was continued until the total time of swimming was three hours. He was then killed at the same time as the control. The total brain and portions from the cervical and lumbar cords were removed. The brains were cut sagittally, and placed in 10 per cent formalin; the other portions were fixed in Held's fluid.

Microscopic examination. In this experiment a large number of sections were cut in series, and a thorough search made for constant differences in staining reaction, amount of chromatic material, size of cells and nuclei, etc. No such constant differences appeared as would go beyond the limits of simple variation. In some slides one might be quite sure of a preponderance of cells of a certain type; for example, showing central chromatolysis; but on actual counting and comparison, the number of such cells will be balanced by an equal number of the same type in the control.

Forced activity

Experiment 5. Dogs. Four young fox terrier dogs of approximately the same size and age were used for this experiment; one served for a control, the other three were subjected to continuous running for periods of one, two and a half, and five hours respectively. They were killed immediately after the exercise, and the nerve tissue from the four animals given identical treatment as to fixation, imbedding, staining, and cutting, the four pieces being mounted side by side in the same block of paraffine, and cut with the same stroke of the microtome knife. In this way the effect of exercise of various grades of intensity could be studied in the cells of the anterior horn of the cord, of the posterior ganglia, and of the cerebellum.

Microscopic examination. Thorough study of all the sections numbering over a hundred, most of which were made in series, was made in this experiment. The various types of cells described by Dolley were particularly kept in mind, and an attempt made to correlate them with various grades of fatigue. Dolley (7) describes thirteen different stages of fatigue corresponding to different grades of work and over-work.

Representatives of practically all these types of cells were found in my specimens, from the resting control animal, as well as from those animals exercised for one, two and a half, and five hours. In order to determine as accurately as possible the relative proportion of these types of cells in the different specimens, a table was made out listing each of these cell types, and then beginning with a section under the microscope, every cell showing a complete nuclear membrane was taken in order, and checked in the proper column. In this way over three thousand cells were counted with the result listed in table 3.

As will be seen in the table, the number of a particular type of cell varies considerably, but this variation is the same for the different animals. There are neither progressive changes in the morphology of the cells from rest to exhaustion nor are there any qualitative or quantitative differences in type of cells from resting and fatigued or even exhausted animals. The animals used in Dolley's experiment exercised at most up to three to four hours altogether. Dolley also describes these types of cells as persisting in the effort to recuperate for from two weeks to several months after exercise no more severe than that of two or three hours running in a treadmill. All these types of cells are admittedly present at the same time (Dolley, *American Journal of Physiology*, vol. 28, p. 151). Dolley describes thirteen stages of fatigue in one animal where the animal exercised one hour in a tread mill. Also, the cells selected by him for illustration of these stages are taken from a single preparation of three sections in the same experiment. Obviously the observations were not over a large enough range of sections nor sufficiently controlled by actual counts of the various types of cells. In the *Journal of Medical Research*, vol. 21, p. 104, Dolley says, "Measurements were made of five cells of each type in five anaemia experiments, one a fatal resuscitation, the other a repeated hemorrhage;" a little farther on, "Measurements were made of ten cells in each of three groups." A great many cells were skipped (those not entire) in Dolley's method of counting, giving a large leeway for a personal factor in the selection of types. *Ibid*, volume 20, page 291, "Measurements were made

TABLE 3
Showing differential counts of 300 cells each

TIME EXERCISED IN TREADMILL	TISSUE	UNCOUNT- ED CELLS	RESTING CELL													
			Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8	Stage 9	Stage 10	Stage 11	Stage 12	Stage 13	
00 Con- trol	Worm.....	97	17	14	18	18	24		21	30	23	14	12	4	8	300
	Lumbar enlarge- ment of spinal cord.....	48	46	32	28	19	39	0	25	20	10	15	10	2	6	300
	Cervical enlarge- ment of cord.....	60	32	24	33	16	33	2	14	30	12	12	18	5	9	300
1 hour	Worm.....	109	20	14	19	16	16	1	19	28	17	13	8	5	5	300
	Lumbar cord. ...	60	38	44	35	18	29	2	15	20	12	8	4	2	3	300
	Cervical enlarge- ment of cord...	88	30	35	22	17	24	1	20	29	1	18	9	5	3	300
2½ hours	Worm.....	97	19	24	16	10	10	2	43	26	14	6	15	8	10	300
	Lumbar cord.....	46	50	38	25	32	30	1	20	27	9	6	5	7	4	300
	Cervical enlarge- ment of cord ...	106	30	20	23	11	21	0	26	15	16	18	9	3	2	300
5 hours	Worm.....	90	32	20	20	30	18	0	22	34	6	4	12	6	6	300
	Lumbar cord.....	53	49	30	26	24	36	0	20	22	11	10	5	7	7	300
	Cervical enlarge- ment of cord.....	65	29	15	27	17	26	3	28	30	12	23	12	6	7	300

Table III, column 3, the uncounted cells as well as those designated under stage 1, 2, 3, etc., are taken from the classification of Dolley, the reference to which has been cited.

of camera lucida outlines of cells and nuclei. This was done in four experiments. . . . Five cells of each type and after each fixative seemed to give a fair average." Dolley's material in which consecutive stages were studied did not receive the same treatment as to fixation, staining, mounting, and cutting. The control and fatigue material was handled entirely separately (Amer. Jour. of Physiol., vol. 25, p. 155). Slight unavoidable variations in the exposure of the tissue to the various agents and different thickness of the cut sections would make such material worthless for comparative study. As pointed out above, this objection cannot be applied to my own experiments, as the handling of material was identical in all cases. Dolley's method of measuring size of cells and nuclei is open to serious objection. In the *Journal of Medical Research*, volume 21, he states, "While not adapted for exact measurement on account of their shape, the largest diameters (cells and nuclei) were always taken." In my own experiments the actual relative areas of the nuclei and cells was computed with the use of the polar planimeter, which traces the entire outline of the cell and gives very accurate results. As seen in the table, I found no difference in the size of the cells of the control and exercise animals.

Electrical stimulation (galvanic)

Experiment 6. Pigeons. In this experiment a pigeon was fastened by the feet in a standing position, while a wire from four 50 amperes galvanic batteries was wrapped around the legs. A current was allowed to pass through the wire once a second by a clock make and break arrangement. With the entrance of the stimulus there was a strong contraction of the leg and thigh muscles. After four hours the contractions became feeble, owing to fatigue, and the pigeon was killed. Rigor mortis of the leg and thigh muscles set in immediately. A control pigeon was killed at the same time.

Microscopic study revealed absolutely no constant morphological differences in the anterior horn cells and in the dorsal ganglion cells from the two birds.

Faradic stimulation

Experiment 7. Faradic stimulation of the sciatic nerve in a cat was applied in this experiment. A cat weighing 2 kilos was anaesthetized with ether, and decapitated according to the method of Sherrington by

tying off the carotids, etc. After a rest of about three-quarters of an hour to give the animal a chance to recover somewhat from the shock, a canula was inserted into the carotid artery, and connected with a manometer for record. The left sciatic nerve was then exposed and stimulated with a faradic current from two dry cells. The secondary coil was placed at eight, later at six. Stimulation was applied at intervals, fifteen seconds stimulation was followed by forty-five seconds rest. Stimulation began at twelve o'clock and continued until five P.M. The heart was still beating strongly at the end of the experiment, the blood pressure remained fair, and reflexes were obtained throughout by stimulation of the sciatic, both in the right and left leg. The contractions on the right were spasmodic, those on the left—the stimulated side, were tetanic. The latter were feeble, and gave all signs of fatigue. The three pairs of dorsal ganglia of the sciatic nerve as well as the lumbar and sacral cord, were removed and placed in the fixing solution. Microscopic examination of the cells and measurements of cells and nuclei from the fatigued and unstimulated side of the cord of the same animal failed to disclose any difference in morphology.

Experiment 8. Faradic stimulation of the sciatic nerve of the frog was used in this experiment, the unstimulated dorsal ganglia cells and anterior horn cells of the unstimulated side of the same animal as well as corresponding material from a second resting frog killed at the same time served as control. The two frogs were pithed and placed in a moist chamber. One-half minute stimulation of the sciatic nerve was followed by one minute rest. The electrodes were applied just above the knee at the back. This interrupted stimulation was continued six hours. The muscles showed response by tetanic contractions. At the end of the period the muscles were still irritable. Three pairs of dorsal ganglia corresponding to the sciatic nerve as well as the spinal cord of the same region were taken for study.

The stimulation produced no changes in the cell morphology that could be detected by a measurement of the size of the cells and nuclei or by study with various powers of the Leitz compound microscope.

Drug stimulation (strychnine)

Experiments 9 to 14. Verworn (13) attributed fatigue in nerve cells in part at least to a local asphyxia of the cells due to an accumulation of fatigue substances and an insufficient supply of oxygen. By perfusing fatigued frogs with oxygenated salt solution, inserting the canula into the aorta, he was able to restore irritability of the nerve cells and corresponding response of the muscles in contraction after they had ceased to respond from fatigue. He succeeded in keeping the muscles and nerve responsive to stimulation for many hours longer than would ordinarily be the case. In experiments 9 to 14 this method was applied in order to continue stimulation and corresponding nerve exhaustion to a degree not possible by the usual methods. Strychnine in doses of $\frac{1}{6}$ to $\frac{1}{3}$ of a grain was given in each case by injecting into the subcu-

taneous area of the lower back just after beginning perfusion with normal salt solution and by adding to the perfusion solution. The heart continued beating throughout the experiment. The strychnine caused violent tetanic contractions at first, which later gave place to continued fibrillary contraction. This served as an indication of the condition of irritability of the spinal cord cells. To prevent direct action of the strychnine on the nerve endings in the gastrocnemius muscle, the femoral artery was tied off. This muscle continued in a state of irritability for several hours. The experiments carried out by this method were continued for a length of time varying from two to ten hours. Controls were used, treated in the same manner, except for the strychnine injections. The subsequent treatment of the nerve material (sections of the spinal cord and from the thoracic and lumbar regions) was identical. There is a striking change in both the control and treated cells, which had been perfused for a long period. Many cells as well as nuclei are swollen beyond the normal size, owing, doubtless, to a simple turgescence caused by a flooding of the circulatory system with the salt solution, and the staining reaction is diffuse. There are, however, no differences in morphological characters between the cells of the strychnine stimulated and the control cells of the spinal cord.

Shock (anaemia)

Experiment 15. A four kilogram dog was given morphine and bled from the jugular vein; 200 cc. were removed, following which the dog remained semi-comatose for five hours. At the end of this time he was killed along with a control dog of four and a half kilograms weight. Material was taken from the cruciate gyrus and cerebellar cortex, fixed in 10 per cent formalin, and stained with polychrome methylene blue. Examination with the microscope revealed no difference in the character of the nerve cells in the shock animal as compared with the control.

DISCUSSION OF RESULTS

It has long been known that prolonged activity of mucous gland cells results in characteristic histological changes in these cells, owing to the disappearance from the cell of certain granules (zymogen granules). This granular material is evidently used to make organic material of the secretion. It was doubtless on the basis of such observations that certain physiologists were led to seek for similar alterations in the highly specialized nerve cell following functional activity. Many of the investigations of these physiologists were confined to one or two experiments, the material was often not sufficiently controlled by normal tissue

for comparison, and frequently the histological technic was faulty. In explanation of the diverse changes described by these workers as resulting from fatigue, it has been assumed that certain materials present in the nerve cells have been katabolized to form energy for the nerve impulse, and that the using up of this material during activity can be detected histologically by the depletion of the granules (chromatic substance), changes in size of cell and nucleus. In a long series of carefully controlled experiments, I could find no evidence of any analogy between the effect of activity in certain glands and activity in nerve cells. In no experiment did the histological structure of the nerve cell following activity show any constant deviation from that of the corresponding resting cells of the controls. Some very sweeping generalizations have been drawn from the conclusions of previous workers; namely, that fatigue, fear, shock and exhaustion may lead to permanent damage and even disintegration of nerve cells. Crile's present theory of surgical shock and of certain aspects of Graves' disease, based essentially on these assumptions, may be cited to show to what extremes these deductions based on insufficiently controlled experiments of this kind have led.

SUMMARY

The effect of various grades of activity on nerve cells was studied in a series of fifteen separate experiments. The animals used were dogs, cats, pigeons, sparrows, frogs, and rats. Every experiment was carefully controlled by a resting animal of the same species, of the same approximate age and size, and the material from both given identical treatment, except for the activity. The nerve cells studied were from the cruciate gyrus, from the cerebellum, from the anterior horn of the spinal cord, and from the dorsal ganglia. In one of the experiments over thirty-five hundred nerve cells classified into thirteen types according to histological characters were counted to determine the relative frequency of characteristics which might be correlated with grades of activity. There was no deviation from the normal in even the most advanced fatigue. Over a thousand cells

and nuclei were measured by computing the areas of the projected outlines with the planimeter. There was found to be no constant difference in size of cells or nuclei resulting from activity. Furthermore, no qualitative differences in histological characters could be found between fatigue and resting nerve cells.

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THE INFLUENCE OF LIGHT AND TEMPERATURE
UPON THE MIGRATION OF THE RETINAL
PIGMENT OF PLANORBIS TRIVOLVIS

LESLIE B. AREY

NINE FIGURES

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INTRODUCTION AND HISTORICAL SURVEY

The pigment granules of many animal cells, which in general are termed melanophores, are capable of undergoing positional changes when adequately stimulated. This property has made possible the development of an interesting field of research, which has had for its goal, the correlation of migratory movements of the pigment with the presence of definite stimulating agents. At first, only the influence of such important environmental factors, as light and temperature, as well as the effects of more artificial stimulating agents like pressure and electricity were sought, and but little attention was given to the rôle played by chemical agents. Recently, however, the possibility of

chemically stimulating certain types of isolated pigment cells has been shown in a striking manner (Spaeth, '13^a; '13^b).

All investigations relating to the physiology of pigment cells may be divided into two general categories, depending on whether body chromatophores or retinal pigment cells serve as the material for experimentation. The general tendency, however, to keep these fields of research sharply separated is wrong, inasmuch as the behavior of the two types of cells has much in common, and it is only by summing our knowledge, after the comparative method, that a thorough understanding of either may be gained.

Light is the commonest and most potent of the natural stimulating agents concerned in pigment migration. In general, it may be said that light induces an expansion, and the absence of light a contraction, of pigment cells.

Photomechanical changes have been demonstrated in the body chromatophores of crustaceans, cephalopods, fishes, amphibians, and lizards. The earliest experimentation showing the effect of light upon the retinal pigment of vertebrates was performed by Kühne ('77) and by Boll ('78), who worked upon the frog. Their results have since been extended upon representatives of the remaining vertebrate classes, although striking movements of the retinal pigment of reptiles and mammals have not as yet been demonstrated. Among invertebrates, photomechanical changes have been found in the compound eyes of insects (Exner, '89), crustaceans (Exner, '91), arachnids (Szczerwinska, '91), and in the eye of cephalopods (Rawitz, '91).

Of the molluscs, the cephalopods constitute the only group in which a response of the retinal pigment to photic stimulation has hitherto been demonstrated. Hensen ('65), upon theoretical grounds, hazarded the guess that the pigment in these animals possessed a certain mobility, for Babuchin ('64) had previously referred to finding the visual rods entirely free from pigment in some specimens of *Sepia* and *Octopus*. It was left for Rawitz ('91) to show that in the light the visual rods of *Sepia officinalis* are pigmented along their whole length, with an especial accumulation at the lens border, whereas in the

dark the pigment is limited to the base of the rod. Chun ('03) confirmed these general conclusions on deep-sea cephalopods, as did Hess ('05) by making a comparison of the pigment responses in pelagic and littoral forms.

Temperature is likewise a controlling factor in determining the ultimate distribution of pigment in many body melanophores. In general (Parker, '06), low temperature has an influence similar to that of light, inasmuch as it favors pigment expansion; high temperature, on the contrary, like darkness, induces pigment contraction.

A similar condition holds for the retinal pigment of certain animals. Congdon ('07, p. 547) found that: "In both *Palaeomonetes* and *Cambarus* the proximal retinal pigment migrates distally when the temperature is lowered and proximally when it is raised." The writer (Arey, 16^a) found an identical tendency in the retinal pigment of fishes. Working with the frog's retina, Gradenigro ('85) first showed that at a temperature of 30°C. the pigment expands, thereby closely simulating the distribution characteristic of light. Later Herzog ('05) confirmed this discovery, and further stated that maximal expansion likewise ensues when the temperature approaches the freezing point, the contracted condition typical of darkness being obtainable only between the temperatures of 14° and 18°C. A reinvestigation of this matter recently made by the writer (Arey, 16^a) has convinced him that Herzog's results are substantially correct and that the behavior of the retinal pigment of the frog to temperature must indeed be considered exceptional; the explanation for this is presumably to be found in the existence of a superimposed nervous control, resulting in the obliteration of the more primitive response.

Positional changes of the retinal pigment of gasteropods in response to definite physiological stimuli have never been recorded. Smith ('06), in his work on the structure of the eyes of pulmonate gasteropods, noticed that the retinal pigment of *Planorbis trivolvis* Say showed processes of variable length, and accordingly he performed a few experiments to determine whether the pigment distribution could be correlated with

corresponding conditions of light and darkness. His results concerning a photomechanical influence were entirely negative, although he states his belief (p. 255) in the existence of an appreciable pigment migration caused by certain unknown factors.

Assuming that the retinal pigment of these animals does undergo migratory movements, it is reasonable to expect that the behavior of pigment cells, having no demonstrable nervous connections (Smith, '06), is dependent upon definite environmental stimuli, hence I decided to reinvestigate the influences that simple stimulating agents such as light and temperature might have upon the retinal pigment of these snails.

MATERIAL AND METHODS

Planorbis was obtained in abundance at certain localities on the bank of the Charles River, Cambridge, Mass. During warm weather it is easily procurable within arm's reach of the shore, but as the water grows colder a gradual withdrawal of animals occurs toward the deeper bed of the river. My experience in collecting during the fall of 1914 will illustrate this behavior. In the second week of October a great wealth of material was discovered at a location where the previous June no specimens had been seen; at the time of this collection the snails were distributed as far up as the water's edge. The next collecting trip was made on November 21, and, in the same spot, where several weeks previously hundreds of animals were obtainable, there now remained a few stragglers only, and these were withdrawn from the bank almost beyond reach. Ten days later scarcely a specimen was left within sight.

I was interested to discover whether this withdrawal was due to an active migration of individuals, or to the effect of the low temperature which might cause the snails to become inactive, whence they would be washed by the current down the sloping bank into deeper water. Mr. W. F. Clapp of the Museum of Comparative Zoölogy informs me that the former of the alternatives is undoubtedly correct. Moreover, low temperature does not necessarily cause these animals to become inactive, since he reports having often broken through the ice

in mid-winter and dredged *Planorbis* from the deeper water to which they resort; under these conditions he has always found them to be in an exceedingly active condition.

This interesting migratory behavior to what is presumably the stimulus of low temperature has a definite 'protective' value to the snail, since an animal thereby removes itself to a depth at which ice is not formed. It is probable that the situation is not one in which an annual rhythm, independent of temperature, has been impressed upon the species, for when *Planorbis*, during its retreat to deeper water, is removed to laboratory aquaria and kept for some time at room temperature it neither manifests a tendency to seek the deepest water, nor does it exhibit negative geotropism. On the other hand, I do not recall that animals introduced into jars of ice water showed any marked tendency to seek the bottom of the dish. It is possible that there is, in fact, a temperature response which, however, comes on but slowly, as evinced by the gradual withdrawal under natural conditions.

Except for minor changes, the following is quoted directly from an enlightening statement made by Mr. Clapp during a correspondence in which I sought his aid in solving the migratory behavior of this animal:

What you write in regard to the migration of *Planorbis* is my idea also. There is little doubt that *Planorbis* has a rest period comparable to that of nearly all other gasteropods—the effect of inactive phases in the life cycle is often clearly marked on the shell. In many species of land shells there are two annual rest periods, the shorter occurring in the summer, the longer in the winter. I have never observed any general inactivity or burrowing during the summer on the part of our New England species of *Planorbis*, and therefore have thought it probable that their rest period occurs during the winter months.

The winter rest period of the land shells in this section of the country is governed entirely by the temperature. When spring arrives early the shells appear early—the hardier species first, the more delicate species later—but all appear earlier than in other years when the warm weather comes on tardily. It seems probable that *Planorbis*, *Physa*, *Lymnaea* and similar shells should follow the same principle, in other words, become inactive and bury themselves when the temperature of the water falls below a definite point. To a certain extent this may be true, but if so, the rest period must be much shorter than in land shells, and, I think, not compulsory as with the latter

forms. My reason for believing this is that I have frequently obtained Planorbis, Lymnaea and Physa, in mid-winter, by breaking through the ice and using a small dredge or net.

Some believe that the fresh water pulmonates do not hibernate at all. That appears to me to be based on negative evidence, since they have not been observed under natural conditions, or rather have been observed under unnatural conditions (vivaria).

At the conclusion of all experiments, to be described subsequently, the animals were beheaded, fixation of the head and the contained eyes occurring under conditions of light and temperature identical with those at which each experiment had been conducted. Perenyi's fixing fluid gave very satisfactory preservation. Sections of 8 μ thickness were cut parallel to the long axis of the eye and were stained with Heidenhain's iron-haematoxylin and with orange-G.

Whenever the experimentation involved the employment of light, strongly diffused daylight, such as is obtainable at north windows, was used.

The microscopical preparations were studied and measurements were made at a magnification of 1400 diameters. In expressing the extent of pigment distribution in numerical terms, two measurements were used. One, which I shall call the 'zonal measurement,' represents the breadth of the band of dense pigment, exclusive of the finger-like processes extending toward the bases of the constituent cells (figs. A, 4 and 5). The second, or 'process measurement,' consists of the zonal measurement plus the average length of the pigmented processes just referred to.

The sense in which a few descriptive terms will be consistently employed needs explanation. 'Peripheral' and 'central' will designate those parts of the eye which are respectively farthest from and nearest to its center (fig. B). 'Distal' and 'proximal,' used in describing retinal cells, are retained in a sense similar to that employed in descriptions of uninvaginated ectodermal cells—hence distal indicates the portion nearest the lens or the lumen of the optic sac, while proximal refers to that part of a cell nearer the connective-tissue sheath at the periphery of the eye (figs. B and C).

DESCRIPTION OF THE EYE OF PLANORBIS

Planorbis is a representative of the group of pulmonate gastropods which is characterized by the possession of but one pair of non-retractile tentacles, at the bases of which the eyes are located. Although the tentacles are not retractile after the manner of an introvert, they nevertheless are capable of exhibiting a high degree of muscular shortening whereby their surface becomes thrown into transverse ridges (fig. A, *ta.*).

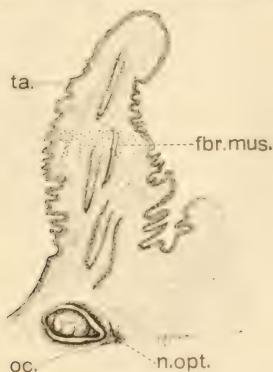


Fig. A An axial section of both the eye and the tentacle of *Planorbis*, showing the position and mutual relation of these structures ($\times 60$). *fbr. mus.*, muscle fibers; *n. opt.*, optic nerve; *oc.*, eye; *ta.*, tentacle.

The eye (fig. A, *oc.*), which lies just beneath the surface epithelium, has the shape of a cone or pear whose base, corresponding to the corneal portion, points outward. The base has a diameter of about $150\ \mu$, whereas the axial measurement is $200\ \mu$.

An axial section of the eye (fig. B) shows the following parts: (1) optic capsule; (2) cornea; (3) retina; (4) optic nerve; (5) lens; (6) vitreous humor.

The optic capsule (fig. 3, *cps. opt.*) is a thin, connective-tissue sheath surrounding the eye, to which the retinal and corneal elements are attached. It is continuous with the sheath of the optic nerve.

The cornea and retina represent differentiated portions of an original sac-like epithelial invagination (figs. A and B). The wall of this closed 'optic sac,' as it is called, never becomes more than one cell thick. The pigment-free corneal cells are only slightly columnar in shape, hence this portion of the optic sac remains as a thin membrane, which is strikingly in con-

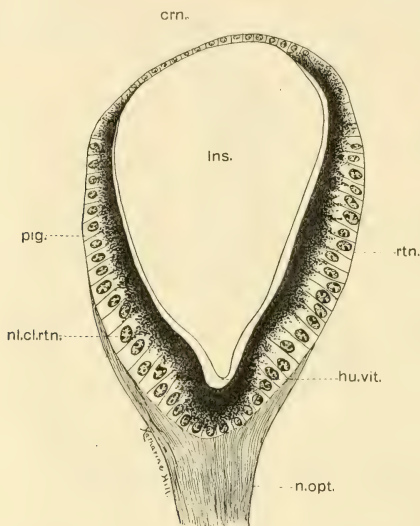


Fig. B An axial section (semi-diagrammatic) of an entire eye of *Planorbis* ($\times 275$). *crn.*, cornea; *hu. vit.*, vitreous humor; *lens.*, lens; *nl. cl. rtn.*, nucleus of retinal cell; *n. opt.*, optic nerve; *pig.*, pigment; *rtn.*, retina.

trast with the thick cornea of some other pulmonates. Its extent is limited to the broad base of the conical eye (fig. B).

The retinal portion of the optic sac is easily distinguishable from the cornea by the presence, in the former, of pigmented cells and by the great elongation of all its elements (figs. B and C). In general, the constituent cells may be said to have a radial arrangement with respect to the optic sac. Notwithstanding the fact that the retina is only one cell thick, it is con-

venient to follow the suggestion of Smith ('06) and distinguish three concentric zones, which, although of an arbitrary nature, are on the whole rather clearly defined. The peripheral zone (fig. C, *rtu. ex.*) is in contact with the optic capsule and is quite free from pigment; it contains the cell nuclei. The pigment of the non-sensory cells is aggregated in a middle or intermediate zone (fig. C, *rtu. m.*); peripherally its limits are ill defined because of irregular processes, which extend down into the cells, whereas centrally a sharp line of demarcation separates the pigment from a third or central zone (*rtu. i.*). The position of the boundary common to the peripheral and intermediate zones is somewhat dependent on the degree of pigment migration under various environmental conditions, which will be discussed in the main part of this paper. The internal or central zone (fig. C, *rtu. i.*) is of constant width and comprises the portion of the retina between the pigmented zone and the lens. It contains the rods (*bac.*)—the photoreceptive elements.

Two kinds of cells form the retinal epithelium, the non-sensory pigmented cells (fig. C, *nl. cl. pig.*) and the unpigmented sensory cells (*nl. cl. sns.*). The pigmented cells, which are the more numerous of the two, are grouped about the sensory cells thereby isolating the latter from each other. These pigmented cells are of two kinds. One set has its nuclei situated close to the optic capsule, while the nuclei of the other set are near the center of the cells and slightly distal to those of the sensory elements. Both types of pigment cells are exceedingly slender, although those with more distally placed nuclei generally have an enlargement just distal to the nucleus; according to Smith ('06, p. 255): "When this part of the cell is free from pigment it appears to contain a vacuole."

The distribution of pigment is variable under different circumstances; the limit of proximal migration in either type of cell, however, is conditioned by the position of the nucleus (fig. C), for pigment is never found proximal to it. The length of the pigment cells, and therefore the thickness of the retina as a whole, varies at different levels in the eye. The thickest portion of the retina is on the sides at a distance of about 60 μ

from the apex of the optic sac. At this thickest region, the distance from the optic capsule to the central zone, that is, the length of the pigment cells, is approximately 35 μ .

The unpigmented or sensory cells (fig. C, *nl. cl. sns.*) are much more robust and have larger nuclei than either kind of pigment cell just described. These cells are of an elongated spindle shape, thickest in the region of the nucleus. The cell body

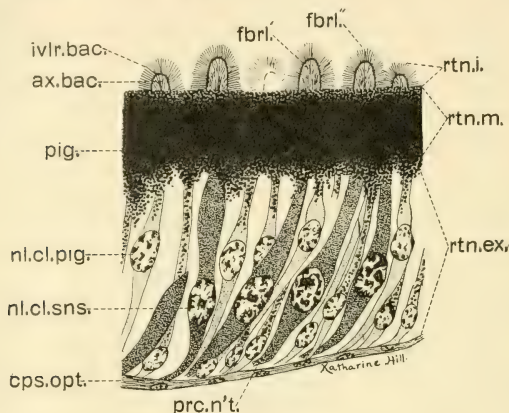


Fig. C A portion of an axial section of a *Planorbis* eye, showing the component retinal elements ($\times 1000$). *ax. bac.*, axis of rod; *cps. opt.*, optic capsule; *fbrl'*, fibrillae of rod-axis; *fbrl''*, fibrillae of rod-mantle; *ivlr. bac.*, mantle (involverum) of rod; *nl. cl. pig.*, nucleus of pigment cell; *nl. cl. sns.*, nucleus of sensory cell; *pig.*, pigment; *prc. n't.*, neurite-process of sensory cell; *rtn. ex.*, peripheral zone of retina; *rtn. i.*, central zone of retina; *rtn. m.*, middle or pigmented zone of retina.

continues through the pigment zone and ends, in the so-called central zone, in a structure known as the 'rod'—this being the photoreceptive portion of the cell. Each rod consists of two parts, centrally a core or 'axis' (*ax. bac.*), and peripherally a radially striate "mantle" (*ivlr. bac.*). Proximal to the nucleus, each sensory cell gives off a neurite (*prc. n't.*), which courses along the inner face of the capsule and is ultimately gathered up with similar neurites to form the optic nerve (figs. A and B,

n. opt.). The optic nerve is covered by a sheath of connective tissue continuous with that of the optic sac. The work of Babuchin ('65), Henchman ('97), and particularly that of Smith ('06) has proved that the sensory cells of pulmonate gasteropods are of fibrillar composition, the terminal brush-like neurofibrils of the rod mantle (fig. *C. fbrl.*') being continuous with the fibers of the rod axis (*fbrl.*') of the cell body, and of the neurite. The length of the rod is 6 μ or 7 μ .

The lens (fig. B, *lens.*) is a large pear-shaped body occupying almost the whole interior of the optic sac. It is a non-cellular secreted mass, the outer portion of which forms a denser capsule or rind.

The vitreous humor (fig. B, *hu. vit.*) fills whatever spaces intervene between the central zone of the retina and the lens, as well as the spaces between the individual rods.

EXPERIMENTAL PART

a. Effect of light and darkness

The initial experimentation consisted in determining whether or not the retinal pigment of *Planorbis* undergoes positional changes, whereby a characteristic distribution is assumed in light and in darkness.

The citation of Smith's statement ('06, p. 255) relative to the tests performed by him on this animal will serve to show both his methods and his results:

In spite of the fact that the rods, lying, as they do, distal to the pigment zone, cannot be protected by pigment migration, as can the rods in cephalopods, the variable position of the proximal region of the pigment suggests the possibility of pigment migration in the eye of *Planorbis*. I therefore attempted to determine by a few experiments whether differing light conditions would produce corresponding changes in the position of the pigment. Several specimens of *Planorbis* were placed for an hour or more in water in a white, porcelain dish which was set in a sunny window. Their heads were then cut off with scissors and fixed in Perenyi's fluid. Sections were made in the ordinary way and stained in Heidenhain's iron-haematoxylin, followed by orange-G. Similar preparations were made from specimens which had been kept in darkness for an hour or more before killing. Comparisons were

then made between the two kinds of preparations in order to learn whether the position of the pigment was different in the two cases. More cases in the 'light' eyes had the pigment reaching quite to the nuclei than in the 'dark' eyes; but there was such a lack of uniformity in different animals, and even in the same retina, that the evidence was not at all conclusive. Repeated experiments did not lead to more definite results. I am satisfied that the pigment does travel up and down in the pigment cells under the influence of some stimulus, but just what is the exciting factor I have not determined. I have not been able to get any evidence of pigment migration in the eyes of either *Helix* or *Limax*.

In another part of the same paper Smith again returns to a consideration of these results and becomes more bold in his inferences. Selected excerpts (pp. 268-269) make plain his final attitude toward the situation in *Planorbis*.

It is probable that pigment movement is a direct response to light-stimulation in *Planorbis* Not having been able as yet to determine the exact conditions under which it occurs, I can only suppose from analogy that the migration is a response to light. The shape of the cells, the position of the pigment in some cells as compared with that in others, and the apparent need of pigment migration in the eye of *Planorbis*, all point to a probable responsiveness of its pigment cells to light;

A personal experience (Arey, 16^a) in determining the lengths of time needed to complete light and dark adaption of the highly mobile retinal pigment of fishes led me to suspect that the 'hour or more' which Smith allowed for the execution of his experiments might be wholly insufficient to permit the photo-mechanical response to proceed to completion. Hence in my experimentation ample time allowances were made for both light and dark adaption.

1. *Effect on normal animals.* Five animals were placed in a battery jar containing water and were kept in a dark-chamber for 24 hours. In the same manner, an equal number of animals were subjected to daylight for 10 hours. A microscopical examination of the resulting preparations gave data as in table 1.¹

¹ The final measurements recorded for each eye represent the mean of the values obtained from several measurements on each side of the retina at a distance of about 50 μ from the entrance of the optic nerve. Experience showed that

TABLE 1

Measurements showing the relative distribution of the retinal pigment of *Planorbis* in darkness and in light at room temperature. The values are mean values expressed in micra, and indicate: (1) the thickness of the main pigment mass (zonal measurement); (2) the zonal measurement plus the length of the pigmented processes (process measurement); (3) the length of the pigment processes obtained by subtracting (1) from (2). The percentage change in each mean measurement in darkness as compared with that in light is also computed

CONDITION OF ILLUMINATION	NUMBER OF RET- INAS MEASURED	MEAN ZONAL MEASURE- MENT	PER- CENTAGE CHANGE IN ZONAL MEASURE- MENT	MEAN PROCESS MEASURE- MENT	PER- CENTAGE CHANGE IN PROC- ESS MEAS- UREMENT	MEAN LENGTH OF FIG- MENT PROCESSES	PER- CENTAGE CHANGE IN LENGTH OF FIG- MENT PROCESSES
		μ		μ		μ	
Light.....	10	11.0	64	16.5	52	5.5	27
Darkness.....	10	18.0		25.0		7.0	

Since the distal margin of the pigment zone—the boundary between central zone (fig. C, *rtn. i.*) and middle zone (*rtn. m.*)—is fixed in relation to each pigment cell, these values show quite decisively that in the light (fig. 2) the pigment migrates distally in the cell, that is, toward the source of illumination, whereas in the dark (fig. 5) the reverse is true. An appreciable thinning out of the pigment in darkness due to its being spread over a greater area, is not demonstrable. It may be that the pigment involved in migration is only that which lies most proximally in the middle zone, or there may occur a general proximal movement, that is, a readjustment of the relative quantitative amounts, throughout the whole mass. Whichever alternative is true, it is certain that the edge of the pigment zone which forms the boundary between the middle and central zones (fig. C) maintains a constant position in relation to the retinal cells.

2. *Effect on excised eyes.* Since light exercises a photomechanical influence on the retinal pigment, the interesting query arises as to whether this effect is direct, upon the cell itself,

restricting the measurements to this most favorable region afforded the fairest numerical representation of the pigment distribution. In these, as well as all experiments to be described subsequently, individuals of *Planorbis* were selected of a more or less uniform size—this precaution will, I think, serve to obviate criticism, if not actual error.

or whether it is accomplished through some nervous mechanism related to the cerebral ganglion. It is true that the probability is against the existence of nervous connections with the pigment cells, since all previous researches have failed to demonstrate structures of this kind, yet it is entirely conceivable that such neuro-fibrils may exist and have hitherto escaped detection; indeed, the extreme difficulty with which the neuro-fibrils of the sensory cells are demonstrated, makes this possibility all the more real. Even if a direct influence of light can be proved, the existence of such neuro-fibrils is not precluded, nor yet their coöperation in effecting the migration of pigment; but it does render this event less likely, and definitely shows, moreover, that whatever may happen in the normal animal, a direct action of light on the pigment cell is a demonstrable phenomenon. If the direct action of light can not be shown to induce migratory movements of the pigment in excised eyes, three possibilities exist: (1) fibers in connection with the pigment cell perform double conduction, transferring afferent impulses to the cerebral ganglion and efferent impulses back to the retina; (2) afferent impulses travel in the optic nerve to the cerebral ganglion while efferent impulses return by hypothetical nerves to the pigment cells, or (3) the optic nerve is of a mixed nature, possessing both afferent and efferent components. Since, however, the existence of double conduction in nerves is a mere postulation, the first of the three possibilities may be safely eliminated.

There is, nevertheless, still another way of viewing the situation, and one that demands serious consideration. The metabolism of an organ isolated from the body to which it belongs is, of necessity, fundamentally altered. Not only is its supply of nutriment cut off, but what is more serious in a short experiment, the elimination of waste is not adequately provided for. If isolated pigment cells do not respond to the direct action of light, the reason may easily be ascribable to an autoanaesthesia caused by the accumulation of the cell's own catabolic products. The probability of this course of events was suggested in some experiments upon the eyes of fishes (Arey, 16^a; 16^b). Moreover, it will be shown in another part of this paper (p. 378)

that anaesthetics are probably capable of exerting an inhibition on the migration of pigment, even in normal snails.

Spaeth ('13^b) found the melanophores on the isolated scale of *Fundulus* to be responsive to ultra-violet rays but not to those of the visible spectrum. Parker ('97), working upon *Palaemonetes*, obtained responses from all three types of pigment cells when excised eyes were brought from light into darkness, or the reverse. This result was observed equally well when stalks containing ganglia were used or when merely retinas, exclusive of ganglia were employed. Hamburger ('89) reported that the pigment of enucleated frog's eyes exhibits migratory movements both in darkness and in light. In another paper (Arey, '16^a) I have described the occurrence of a pigment migration when the previously dark-adapted excised eye of the common horned pout, *Ameiurus*, is brought into the light; in the reverse exposure (light to dark), however, no response ensues; furthermore, in several other fishes which were studied, even the direct influence of light could not be shown.

Experiments on *Planorbis* were made upon animals that had been adapted to light or to darkness for 6 and 24 hours, respectively. Such animals were beheaded and small pieces of tissue, bearing a tentacle, were then placed in a watch glass containing an abundance of Ringer's solution and subjected to light or to darkness according to desire. The exposure to light lasted 4 hours, whereas in the dark, eyes were left for 5 hours. Table 2 summarizes the results of these determinations.

The results of both these sets agree very closely with the values given previously for normal light-adapted eyes (11.0 μ and 16.5 μ for zonal and process measurement respectively). The obvious conclusion to be drawn from these experiments is that light has a direct influence on a previously dark-adapted pigment cell, but that light-adapted retinas do not change in darkness. The direct action of light on the retinal pigment of this animal, therefore, is identical with that of *Ameiurus*.

I am inclined to interpret these results in the following way. The conditions under which an isolated eye is placed undoubtedly favor the accumulation of catabolic products. This unremoved

TABLE 2

Measurements showing the relative distribution of pigment in the excised eyes of *Planorbis* in darkness and in light. The values are mean values expressed in micra and indicate: (1) the thickness of the main pigment mass (zonal measurement); (2) the zonal measurement plus the mean length of the pigmented processes (process measurement); (3) the length of the pigmented processes obtained by subtracting (1) from (2). The percentage change in the mean zonal measurement in darkness as compared with that in light is also computed

CONDITION OF ILLUMINATION	NUMBER OF RETINAS MEASURED	MEAN ZONAL MEASURE- MENT	PERCENTAGE CHANGE IN ZONAL MEASURE- MENT	MEAN PROCESS MEASURE- MENT	MEAN LENGTH OF PIGMENT PROCESSES.
		μ		μ	μ
Dark-adapted eyes sub- jected to light.....	6	11.0	14	14	3.0
Light-adapted eyes sub- jected to darkness.....	6	12.5		14	1.5

waste exerts an inhibitory or anaesthetic influence upon the normal activity of the protoplasm of the pigment cells. Since in the case of the retina it is even probable that light favors catabolism, it follows that the movement of the retinal pigment in the light only, must be due to the greater efficiency of light as a stimulating agent. The strong stimulatory effect of light, therefore, is able to break through the protoplasmic inhibition caused by accumulated wastes, whereas darkness is ineffective in this respect. If this reasoning is tenable, one may infer that movements of the pigment would also be realized in darkness, provided artificial circulation could be maintained. That light actually is a more efficient stimulus than darkness, is further suggested from the determinations of adaption times (*vide infra*).

(b) *Determination of adaption times*

It is evident from the foregoing observations that photo-mechanical changes do occur in the pigment of the *Planorbis* retina, and furthermore, one is led to suspect that the indecisive results obtained by Smith ('06) are assignable to the short duration of his experiments, which, in his own words, lasted but "an hour or more." Hence the pertinent inquiry as to

the exact periods of time necessary to complete light- and dark-adaption becomes the next object of consideration.

Snails which had previously been thoroughly adapted to light (fig. 2) or to darkness (fig. 5), were subjected to opposite conditions of illumination. Individuals were removed and killed at half-hour intervals until the experiments had continued for 5 hours. Microscopical examination of the retinas gave the following results:

- Dark-adapted *Planorbis* subjected to light:
 - Incomplete light-adaption occurred in 3 hours
 - Complete light-adaption occurred in 4 hours
- Light-adapted *Planorbis* subjected to darkness:
 - Incomplete dark-adaption occurred in 4 hours
 - Complete dark-adaption occurred in 5 hours

Our suspicion as to the cause of Smith's failure to procure satisfactory and consistent results is corroborated, since the time allotted for his experimentation was quite inadequate.

From the comparative standpoint, it is interesting to see how the rapidity of pigment migration in *Planorbis* agrees with corresponding movements in the eyes of other animals.

In the compound eyes of the prawn, *Palaemonetes*, Parker ('97) gives the following data for the time consumed in the adaption of the proximal, or retinal, pigment:

Darkness to light.....	30 to 45 minutes
Light to darkness.....	45 to 60 minutes

The writer (Arey, 16^a), working upon several fishes, has shown that the complete adaption of the retinal pigment requires longer periods of time than have generally been supposed. These determinations may be combined in the following summarization:

Darkness to light.....	45 to 60 minutes
Light to darkness.....	30 to 60 minutes

An interesting comparison with the cephalopod type of eye is made possible through the values determined by Hess ('05), from whose work it appears that many hours (48 or more) are

necessary for dark-adaption, whereas a much shorter time (approximately $1\frac{1}{2}$ hours) suffices for light-adaption. Hess's results upon *Octopus defilippi* (one of the several species studied by him) are especially astonishing, since he states that dark-adaption occurs in 48 hours, whereas light-adaption may be practically completed in 15 minutes.

(c) *Effect of temperature*

Next to light, temperature is the commonest environmental stimulating agent that controls the movement of retinal pigment. Accordingly, a series of tests were made, both on normal and excised eyes, to discover whether a correlative temperature response is demonstrable.

TABLE 3

Measurements showing the relative distribution of pigment in the eyes of Planorbis at 3° and 32°C. both in darkness and in light. The values are mean values expressed in micra and indicate both the thickness of the main pigment mass (zonal measurement), and the zonal measurement plus the length of the pigmented processes (process measurement). Corresponding values at room temperature, copied from Table 1, are given in parenthesis

CONDITION OF ILLUMINATION	NUMBER OF RETINAS MEASURED	TEMPERATURE	MEAN ZONAL MEASUREMENT	MEAN PROCESS MEASUREMENT
		<i>deg. C.</i>	μ	μ
Light.....	6	3	10.0 (11.0)	14.0 (16.5)
Light.....	6	30	12.5	19.0
Darkness.....	4	3	13.0 (18.0)	19.0 (25.0)
Darkness.....	6	32	14.0	24.0

1. *Effect on normal animals.* Fully light- and dark-adapted snails, retained in jars of water, were each subjected to temperatures of approximately 3° and 32° C. At the end of 4 hours the eyes were fixed at temperatures identical with those at which the respective experiments had been conducted. The data from these determinations are presented in table 3.

These data show that temperature is extremely efficacious in evoking positional changes in the retinal pigment. Since,

both in the light and the dark, the pigment shows greater distal migration at low (figs. 1 and 4) than at high temperatures (figs. 3 and 6), it follows that low temperature and light on the one hand, and high temperature and darkness on the other, tend to influence pigment migration similarly. As the data show (this being a logical corollary of the proposition just stated), extreme distal migration is favored by the coöperation of light and low temperature (fig. 1), whereas an extreme proximal migration is best obtained at high temperatures in the dark (fig. 6). It is pertinent to mention in this connection that the pigmented processes at the higher temperatures in the dark (figs. 5 and 6) were very thick as well as long.

It will be noticed that in the light the values given in parenthesis (representing the results at room temperature copied from table 1) are intermediate between the measurements at the extreme temperatures: in the dark, on the contrary, this progressive relation does not hold.

TABLE 4

Measurements showing the relative distribution of pigment in the excised eyes of Planorbis at 2° and 30°C. in the light. The values are mean values expressed in micra and indicate both the thickness of the main pigment mass (zonal measurement), and the zonal measurement plus the length of the pigmented processes (process measurement)

CONDITION OF ILLUMINATION	NUMBER OF RETINAS MEASURED	TEMPERATURE	MEAN ZONAL MEASUREMENT	MEAN PROCESS MEASUREMENT
		deg. C.	μ	μ
Light.....	3	2	9.6	10.2
Light.....	3	30	10.3	17.5

2. *Effect on excised eyes.* A few experiments were performed to determine whether the temperature response is obtainable in light-adapted eyes which are isolated from the body. In all respects the method of experimentation was identical with that employed when normal animals were used.

Table 4 gives the results from the small number of retinas measured, an accident having destroyed the remainder of the material.

The results thus tabulated are so similar to those just discussed in the case of normal animals that comment is scarcely necessary. The specimens used were rather undersized and this probably accounts for the low values obtained in each set.

Unfortunately no material was available when temperature experiments upon dark-adapted excised eyes might have been performed. Had an influence of temperature been proven under these conditions it would have been of considerable interest, inasmuch as darkness was found (p. 371) to be ineffective upon excised eyes which had previously been subjected to light. This result was indeed realized in my work (Arey, '16^a) upon the isolated eyes of fishes, thereby suggesting that temperature is a more efficient stimulating agent than is light.

(d) Effect of anaesthetics

In a few instances only, have clear cut observations been recorded as to the anaesthetic action of definite substances upon retinal pigment cells. Ovio ('95) and Lodato ('95) agreed that cocaine arrested pigment migration in the frog. The writer (Arey, '16^a) has found that carbon dioxide or ether, without permanently injuring the retinal pigment cells of fishes, is capable of arresting completely the movements of the pigment in light as well as in darkness. Chloretone and urethane, on the contrary, although of sufficient strength to kill the animals as organisms, did not prohibit pigment migration. Since 5 per cent solutions² of carbon dioxide effectively controlled these migrations, it was suggested that this, the commonest of catabolic products, might well be the effective agent in preventing migratory movements of the pigment in excised eyes.³ It is possible that the results, already presented, in which it was found that the pigment of the isolated eyes of *Planorbis* migrated in light but not in darkness, may be interpreted in a similar manner.

² No attempt was made to ascertain the minimal concentrations which would accomplish this end.

³ Of four fishes used, *Ameiurus*, *Abramis*, *Carassius*, and *Fundulus*, the retinal pigment of the excised eyes of *Ameiurus* changed its position in the light, whereas in none of these species did a change occur in darkness.

A few experiments seemed to indicate that both ether and carbon dioxide did completely check the pigment movements when *Planorbis* was introduced from darkness into light, yet the snails were very susceptible to the anaesthetics and in no case survived. By careful experimentation it might be possible to find concentrations at which movements of the pigment would be prevented, yet the pigment cells and the animals (as determined by controls) survive. After several trials, in all of which the snails did not outlive the 3 to 4 hours subjection to the anaesthetics necessary for light adaption, the work was discontinued.

It is evident that the chief value of the few determinations made lies in their suggestiveness and not in the actual results gained, which, because of the absence of appropriate controls, are properly open to criticism. Hence one may be allowed merely to suggest that the pigment in the excised eyes of *Planorbis*, similarly to that in *Ameiurus*, is able to overcome the probable anaesthetic effect of accumulated wastes in the light only, whereas the weaker response in the dark fails to appear at all because of the presence of the same catabolic products.

DISCUSSION

In 1906 Parker analyzed the results of many workers concerning the influence of light and temperature upon melanophores and stated his conclusions in the following generalization (p. 413): "It is probable that in all melanophores in which there is a migration of pigment, light or low temperature will induce a migration toward the source of illumination and the absence of light or a high temperature a migration in the reverse direction." Certain cases, however, may be cited which do not conform to Parker's dictum. Such reversed behavior to light is exhibited by the melanophores of the frog (Harless, '54), the eel (Steinach, '91) and Triton (Hertel, '07). A lack of conformity is likewise shown in the temperature responses of the frog's retinal pigment (Herzog, '05; Arey, '16^a). It is not improbable that, in these animals, a more or less active nervous control

has been superimposed upon the more primitive direct melanophore response, thereby making the behavior appear anomalous.

It is evident, however, that the results enumerated in this paper relative to the influence of light and temperature upon the retinal pigment of *Planorbis*, are conformable with the previously quoted generalization of Parker. The existence of a similar agreement in the behavior of the retinal pigment to temperature was found by Congdon ('07) upon the prawn, *Palaemonetes*, and by myself (Arey, '16^a) upon several fishes.

The rôle played by body chromatophores in the economy of an animal is supposedly adaptational with respect to its environmental coloration, and perhaps the regulation of its body temperature as well. The significance of movements of the retinal pigment, on the contrary, are by no means patent, and the agreement of its responses to light and temperature with those of melanophores in general is of considerable speculative interest. Is the reason for this unanimity of response a phylogenetic one, retinal pigment cells retaining a primitive behavior? Or is it merely the similar but discontinuous expression of common physiological needs? Or is the agreement purely fortuitous, not involving the fulfilment of any common need? Or is the influence of light and temperature upon the pigment-containing protoplasm necessarily similar wherever mobile pigment cells are found, the pigment migration under any condition (when not controlled by the nervous system), therefore, being always predictable? By allowing one's fancy free rein, numerous other possibilities may be conceived.

Since we know many more instances of immobile than of mobile pigment cells in the tissues of animals, it is reasonable to suppose, and, moreover, evolutionary doctrines compel us to assume, that the existence and perpetuation of the latter type of cells is not without significance. Thus, ignoring all questions as to the reason for this or that behavior of pigment cells, we at least can safely assume with Parker ('06, p. 411): " . . . it might be supposed that if a case arose in which a reversed migration of pigment would be of service to the organism, such form of migration would be evolved and a set of pigment cells

in which the pigment granules under illumination would migrate away from the source of light instead of toward it would be produced."

It has already been pointed out that the exceptional behavior of the body chromatophores of the frog, eel, and Triton probably is the result of a nervous control. As far as direct responses are concerned, Parker's further assertion ('06, pp. 411-412), for all we know to the contrary, is true: "Hence it seems probable that the melanophores, retinal pigment cells, and other like structures in which dark pigment granules exhibit migratory movements, are restricted as to these possibilities, and that in light they always transport their pigment toward the source and never in the reverse direction."

Since the pigmented cells of the gasteropod eye have no demonstrable nervous connections, this condition, if true, renders these cells wholly indifferent in the process of light perception. It follows, therefore, that such cells are not comparable to the pigment-bearing reticular cells of certain arthropods in which the pigment granules, contained within the sensory cells themselves, change position in light and in darkness. The situation in the gasteropod, however, is quite similar to that in the vertebrate eye in this respect.

In several crustaceans (e.g., Parker, '99, upon the amphipod, *Gammarus*) it has been shown that in darkness the pigment of the reticular cells moves proximally, thereby leaving part of the rhabdome devoid of pigment, whereas in light the rhabdome again receives a pigment sheath. These responses were interpreted as having the following significance. In the light, the rhabdome, surrounded by pigment, is protected from overstimulation by light reflected internally from the white pigment; in dim light, on the contrary, the efficiency of the visual apparatus is increased by the withdrawal of pigment, whereby the reflecting mechanism enables the eye to make the best use of the available diffuse light. Theories involving the principles of overstimulation as well as of optical isolation have also found many supporters among those who attempt to explain the phenomenon of pigment migration in the vertebrate eye (Arey, '15).

The interpretation of migratory movements in the retinal pigment of gasteropods, or to go further, the interpretation of the presence of such pigment at all, from the standpoint of preventing overstimulation or of securing optical isolation, involves greater difficulties than is the case in arthropods. In the snail, the brush-like rods, which have been described as the photo-receptive portions of the sensory cells, are entirely distal to the pigment zone, hence these elements are at all times exposed to the full strength of light. It has been pointed out, however, that the pigment may serve as a background to prevent reflection and thus it indirectly produces a limited 'optical isolation.'

The accumulation of pigment far from the cell bases, and the slight extent to which it can be induced to move proximally, suggests that its chief utility may exist in a relationship with that portion of the sensory cell which it immediately surrounds. In this connection, a statement by Smith ('06, p. 270) is suggestive:

Aside from preventing internal reflections within the rod zone, it is possible that the pigment is directly protective to that part of the visual cell which is surrounded by it. We do not know that the middle part of the sensory cell is not sensitive to light. Neither do we know how or where light vibrations are transformed into nervous impulse. If the transformation takes place in the middle zone, the pigment may serve some purpose there.

Having thus seen that the presence of retinal pigment of gasteropods is only to be interpreted with difficulty, how much greater is the task of assigning explanations to the feeble movements exhibited by this pigment. It may be said that explanations of the presence of retinal pigment and of its movements are self-inclusive, an explanation of one necessarily involving the other. That presence and mobility represent two more or less distinct factors, follows, however, from the fact that in some, and perhaps in most gasteropods (e.g., in *Helix* and *Limax*, Smith, '06) the retinal pigment is non-motile.

Theorists who have viewed the migratory movements of retinal pigment in those eyes where striking changes occur as indicative of the prevention of overstimulation or the procural

of optical isolation, have likewise associated a retreat of the pigment in dim light with the presence of a more diffuse and weaker photic stimulation, but, withal, a stimulation which, under the circumstances, thereby rises to its highest efficiency. An assumption, however, is involved in this reasoning, for it must be shown that the pigment really does retreat in dim light, as, in truth, it does in darkness. This essential point, however, has too often been ignored.

Furthermore, if the distribution of pigment in twilight were essentially similar to that in bright light, it follows that explanations which attempt to solve the meaning of the position assumed by the pigment in light, only answer half of the questions involved, for it may well be asked—Why should there be an extensive movement in total darkness, or why, since a permanently expanded condition would seem to be all that is required, any movement at all? These queries merely show that easily devised explanations, as for example those based upon the theory of optical isolation, may not touch the primary reason at all—such obvious relations may have a secondary importance or may even be purely fortuitous.

Returning to the case under consideration, the movements of the retinal pigment of *Planorbis*, through the influence of light, are very limited in comparison with those in many animals. The dispersion in the compacted pigment mass, and the formation of sparse granular processes, do not visibly alter the density of the main pigmented zone. It is difficult to see (assuming that a proximal migration does occur in dim light as well as in darkness) how these changes would be of any great value to the animal in the ways in which the pigment commonly has been supposed to act. Even the vague assumptions of a nutritional relation of the pigment to adjacent sensory cells is discrepant with certain phases of the photo- and thermo-mechanical changes.

In the body chromatophores of certain animals, e.g., lizards, the response of the pigment to light and temperature may be of use in regulating the body temperature of the animal. Thus (quoting from Parker, '06, p. 411): "The dark color of the lizard's skin in moderate illumination at a moderate temperature

insures, possibly, among other things, a certain degree of warmth which would be superfluous, if not dangerous, at a higher temperature, and in consequence the skin becomes light-colored in hot sunlight."

The significance of temperature responses of the retinal pigment of *Planorbis* is even more obscure than the meaning of photic influences. It is easy to construct several groundless hypotheses to explain why low temperature, acting similar to light, and high temperature, acting similar to darkness, could be of advantage to the snail in the regulation of its retinal economy. Such interpretations, however, possess even less value than the speculations concerning the rôle played by light.

To devise an explanation from the adaptional standpoint that will account for the temperature responses occurring in total darkness would seem a hard task. It may be, however, that the cell thus responds to temperature stimulation because the change is of use in the light-adapted eye only, whereas in darkness the similar response to similar stimulation is gone through with perfunctorily, as it were, even though it is of no use to the organism.

The cautiousness of the following position, with the formulation of which this discussion will be closed, may be its only commendation; yet when our absolute ignorance of fundamental facts is considered, caution may well qualify as a cardinal virtue. An analysis of the conditions of pigment migration in the various vertebrate classes has led the writer (Arey, '15; '16^a) to an identical conclusion for these animals as well.

Recognizing, therefore, that the movements of the retinal pigment of *Planorbis*, as well as of other animals, to light and to temperature may have an adaptive significance, and, furthermore, realizing that (with the possible exception of arthropods) we at present are quite unaware of the meaning of these movements, it would seem that we are permitted to indulge only in interpretations formulated in terms of protoplasmic responses to definite stimulating agents and that the questions of utility thereby involved must await the establishment of a more thorough knowledge of the co-existing factors.

SUMMARY

1. Light causes the retinal pigment of normal *Planorbis* to migrate distally (toward the source of illumination). Darkness causes a proximal migration.

2. About 4 hours is necessary for the pigment to assume the distribution characteristic of light-adaption, whereas about 5 hours is demanded in accomplishing the reverse process of dark-adaption.

3. The pigment of dark-adapted, excised eyes exhibits migratory movements when exposed to light, and is, therefore, capable of direct stimulation. In the converse treatment (light to dark) of excised eyes, on the contrary, no change in the position of the pigment occurs. It is possible that the absence of positional change in darkness is indicative of an anaesthetic effect, produced by the unremoved products of catabolism, the more vigorous influence of light, however, being able to overcome this inhibitory tendency.

4. High temperature (30 C.) induces proximal migration of the normal retinal pigment both in darkness and in light. Low temperature (3 C.) induces distal migration both in darkness and in light.

5. Upon light-adapted excised eyes, low temperature likewise favors proximal migration and high temperature favors distal pigment migration.

6. *Planorbis* affords the first case among gasteropods in which the occurrence of positional changes in the retinal pigment has been correlated with the presence and absence of light. For the first time among molluscs, a thermo-mechanical influence upon the retinal pigment has been demonstrated.

7. It is probable that low concentrations of anaesthetics, such as carbon dioxide or ether, are capable of completely arresting migratory movements of the *Planorbis* retinal pigment. This suggests that carbon dioxide, as a product of catabolism, may be the inhibiting factor that prevents migratory movements of the pigment when excised eyes are brought from light into darkness.

8. The adaptional significance of the existence of mobile pigment in the retina of Planorbis is at present quite obscure. These movements can only be correlated with the presence of known environmental conditions, and interpreted in terms of protoplasmic responses to definite stimulating agents.

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December 6, 1915

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PLATE 1

EXPLANATION OF FIGURES

eps.opt., optic capsule
pig., pigment

rtn.ex., peripheral zone of retina
rtn.i., central zone of retina

The figures of this plate, taken from axial sections of the *Planorbis* eye, are photographs at a uniform magnification of 540 diameters and show the distribution of retinal pigment at various temperatures in light and in darkness.

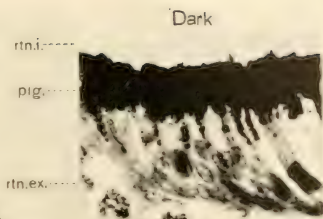
- 1 At 3°C. in the light.
- 2 At room temperature (21°C. \pm) in the light.
- 3 At 30°C. in the light.
- 4 At 30°C. in the dark.
- 5 At room temperature (21°C. \pm) in the dark.
- 6 At 32°C. in the dark.



1

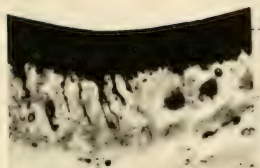
pig.
cps.opt.

3°C.



rtn.i.
pig.
rtn.ex.

4



2

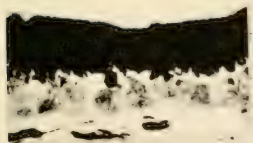
pig.
rtn.ex.

ca. 21°C.



pig.
rtn.ex.
cps.opt.

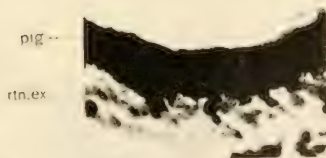
5



3

pig.
rtn.ex.
cps.opt.

ca. 31°C.



pig.
rtn.ex.

6

ABSENCE OF CHROMATOLYTIC CHANGE IN THE CENTRAL NERVOUS SYSTEM OF THE WOODCHUCK (*MARMOTA MONAX*) DURING HIBERNATION

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SIX FIGURES (TWO PLATES)

INTRODUCTION

Since the discovery by Nissl in 1885 that the chromophilous granules in the cytoplasm of nerve cells—granules which had already been described by Flemming ('82) and which von Lenhossék later termed *tigroïdes*, though now more generally spoken of as Nissl granules—are intensely stained by basic aniline dyes, especially by methylene blue, the disposition of these granules, under numerous physiological and pathological conditions, has been the subject of much study. From the extensive functional alterations that numerous authors (Valentin, Dubois, Merzbacher, etc.) have reported to occur in the nervous system during hibernation, and from the morphological variations which are said to take place in the nerve cells in certain other conditions, such as sleep and starvation, one would naturally expect to find marked changes, especially in the Nissl granules, during hibernation. This state, as is well known, is attended in some animals by almost continuous sleep and profound torpor for four months and even longer, during which time no food whatever may have been eaten and the body temperature has been reduced to but a few degrees above the freezing point.

HISTORICAL

An examination of the literature revealed the fact that some observers have reported marked structural changes in the nerve cells during hibernation. Levi ('98) in the toad (*Bufo vulgaris*)

found that the Nissl bodies had greatly diminished in size and the acidophil granules had become basophilic during winter-sleep; but in hibernating mammals no changes were observed. A more detailed study by Legge ('99), however, led this author to conclude that the cells of the cerebro-spinal axis of hibernating bats do undergo visible changes. In the cells of the cerebral cortex he found the Nissl granules to have elongated, being more fusiform in shape, and to be displaced towards the periphery, forming a sort of envelope to the cytoplasm. Baroncini and Beretta ('00) also reported morphological changes in the spinal cord and especially in the cerebral cortex of mammals (muscardin and bat) during winter-sleep. They found that the Nissl granules had greatly decreased and stained more diffusely. The chromatic substance of the nucleus was also more diffuse than in active specimens and the nucleolus seemed to have disappeared in many cases. Essentially the same changes in the Nissl granules in the cells of the cerebro-spinal axis and in the Purkinje cells of the cerebellum of hibernating hedgehogs, were reported by Marinesco ('05) who found a distinct decrease in these granules during the torpid state. Those that remained were reduced to fine granules or were diffused in the cytoplasm. A more recent study by Zalla ('10) agrees with the older results obtained by Levi in regard to mammals. Zalla found no appreciable morphological difference in the Nissl substance in the dormouse (*Myoxus glis*) during hibernation as compared with the active state. His results in amphibia were not constant, but in reptiles he found a distinct decrease in this substance in the motor cells of the cord and pons during winter-sleep.

The question of changes in the chromophilous substance of the nerve cells during hibernation in mammals thus seems to be unsettled. Legge, Baroncini and Beretta, and Marinesco apparently have observed marked changes, while Levi and Zalla could not establish any such chromatolysis. However, these authors did not all work on the same species of mammal. In amphibia there is also some disagreement. Levi found a distinct decrease in the Nissl granules during hibernation, while Zalla, whose results were not very constant, believes that there

are no changes. In reptiles, however, Zalla did find a marked decrease during torpor.

Some other morphological changes in the nerve cells during hibernation may be briefly mentioned. Querton ('98) by means of the Golgi method on cerebral neurones found that the protoplasmic prolongations retract and assume a moniliform appearance during the dormant period. This, however, could not be substantiated by Baroncini and Beretta ('00). Some work has been done on the neurofibrillae. Tello ('03) reported that in the motor cells of the spinal cord of lizards the neurofibrillae are much thicker than usually found. Tello and Cajal ('04) found that this was true only during the cold season when the animal is dormant, and that when the animal is warmed up and made active the fibrillae become more numerous and much finer. Cajal thus believes that the hypertrophy of the neurofibrillae is due to the cold and resulting diminished spinal reflexes, because exposure of the animal to a low temperature brought about this giantism of the neurofibrillae while warming it up caused a return to the normal, and because this change is not seen in the telencephalon and mesencephalon whose cells retain their activity to a much greater extent during the lethargy. Marinesco ('05) has repeated these experiments on young cats and dogs and Dustin ('06) has done the same on young rabbits with essentially similar results. A temperature below $10^{\circ}\text{C}.$, however, was found to be less effective than $10^{\circ}\text{C}.$ in bringing about these changes in the neurofibrillae, according to Marinesco. This may be related to the fact that a temperature too low excites hibernating mammals and finally wakes them up since the body temperature rises as a result of increased activity. The latter author found no such modification of the fibrillae in hibernating hedgehogs, which fact he interprets as indicating that the activity of the nervous system of hibernating mammals is not reduced to the extent that it is in the lizard and other cold blooded animals. Zalla ('10), on the other hand, found that the neurofibrillae were fewer and farther apart in the dormouse during hibernation.

PRESENT INVESTIGATION

In view of the conflicting reports as just reviewed and the more recent observations by Crile on changes in the brain cells under various emotional and other conditions, much less striking than the phenomenon of hibernation, the work we have done on the woodchuck in this regard, seems worthy of a brief note. Woodchucks, or ground hogs, which represent the American marmots, are some of the best examples of hibernating mammals in this country. All species remain dormant for four to six months each year, and hence constitute good material for a study of hibernation. This work was commenced early in January 1913 by J. A. Myers, who fixed and imbedded the central nervous system of six woodchucks, four of which were killed at various intervals while hibernating (January 18, February 6, March 15 and April). One was killed shortly after waking up (March 15) and another, during the following summer.

In addition to studying the above series by means of the Nissl stain, the other co-author prepared another series consisting of the brain and spinal cord of fifteen woodchucks killed during the autumn, winter and spring of 1913-1914. This series includes one animal killed about a month before hibernation (October 25), one just before hibernation (November 22), five during hibernation (February and March)—one of these was partly awake when killed on February 16, but was sluggish and had a rectal temperature of 19°C .—one within two days after waking up (March 16) and seven others which had been awake from three days to more than a month. Three of this last group had been fed for one, two and three weeks respectively. These animals were kept in the artificial burrows which were designed by Professor Simpson of this laboratory and which have already been described elsewhere.¹ The rectal temperature of the dormant animals varied from 8°C . to 12°C ., whereas, the temperature of the active animals ranged from 32°C . to 38°C .

¹ Rasmussen, A. T., Amer. Jour. Physiol., 1915, vol. 39, p. 20.

All the animals of both series were killed quickly by transfixing the heart through the chest wall. No anaesthetic was used, except in five cases where only sufficient ether was given to keep the animal quiet. These five animals were all killed after hibernation while awake and active. The amount of ether given did not seem to have any noticeable effect on the Nissl granules. If, however, these five cases are excluded from consideration because of the introduction of this additional factor, the two series involve as strictly comparable cases two before hibernation, nine during hibernation and five after hibernation. The blood was washed out immediately after death with normal saline solution by injection through the aorta. The saline was followed by a saturated aqueous solution of bichloride of mercury to which had been added 10 per cent of formalin. Thus the central nervous system was fixed very quickly *in situ*. The whole brain and cord were then removed and cut into transverse sections a few millimeters thick. The desired levels were further fixed in a saturated aqueous solution of bichloride of mercury for 48 hours, washed in running water 36 hours, and dehydrated in graded alcohols containing iodine in the usual manner. The tissue was cleared in xylol and imbedded in paraffin melting at 54°C. The levels thus imbedded were: olfactory bulb, motor cortex, mid-thalamus, midbrain at the level of the superior colliculus, mid-cerebellum and pons, medulla oblongata at extreme inferior border of fourth ventricle, first cervical, sixth cervical, sixth thoracic, second lumbar and lower lumbar segments of the spinal cord.

Sections were cut five microns in thickness, except in the case of the spinal cord where the sections were six microns thick, and stained on the slide for ten minutes in a large quantity of hot (70°C.) solution of 1 per cent methylene blue in water saturated with aniline oil. The excess stain was washed off rapidly in water and decolorization carried on by transferring the slides directly to 95 per cent alcohol for two to ten minutes. Dehydration was completed in absolute alcohol and clearing in cajuput oil followed by xylol. The sections were mounted permanently in Canada balsam dissolved in xylol. Where the size

permitted, corresponding sections from all animals of a series were fixed on the same slide to insure equal staining. In other cases several sections from the same block were placed on one slide and all the corresponding slides stained together by carrying them through the reagents by means of a basket, or rack, which would contain the entire lot.

RESULTS

The nerve cells of the woodchuck are essentially typical, containing the usual large round nucleus, with one nucleolus. The chromophilous substance in the cytoplasm has the usual appearance and arrangement so well known that no description will be necessary here. In spite of all precautions there are noticeable variations in the size, distinctness and arrangement of the Nissl bodies in homologous cells of animals in the same state and even in the cells of the same group in a particular section. These variations are found in both dormant and active animals. We can detect no modification in the Nissl granules characteristic of the hibernating as compared with the non-hibernating state. Certainly in these woodchucks there is not the difference indicated by the figures given by Marinesco in the case of the hedgehog. The chromophilous substance is present during hibernation in at least as great a quantity as at other times and presents the usual appearance when stained with methylene blue. The arrangement of the granules varies somewhat even in cells of the same group, being more abundant in the periphery of the cells in some cases and in others being grouped more densely around the nucleus. The size and shape of the bodies vary from fine irregular granules to larger elongated ones; but when a large number of cells are examined the extreme variations may be found in animals in the same state and often in the same section. A predominance of a particular variation in either state can not be established. The accompanying figures and explanations will suffice to indicate the general cell picture before, during and after hibernation. The larger types of nerve cells were selected as illustrations because in them the Nissl bodies are more distinct and make better photographs.

We wish to thank Prof. Sutherland Simpson for his helpful guidance in this research. We are also indebted to Prof. B. F. Kingsbury for assistance in taking the photomicrographs.²

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² In regard to the general problem of the correlation of structural changes to activity in nerve cells, attention should be called to the experiments carried out on six different species of animals by R. A. Kocher, reported in this journal (vol. 26, No. 3, p. 341) since this article was submitted for publication. This author could find no correspondence between the size or structural characteristics of nerve cells and various grades of activity.

PLATE 1

EXPLANATION OF FIGURES

The three photographs in this plate were taken from the nucleus hypoglossus. The sections from the various animals were mounted on the same slide and hence stained together. $\times 320$.

1 Before hibernation. Woodchuck killed October 25, 1913, without any anaesthetic. Animal active. Rectal temperature 37.6°C .

2 During hibernation. Woodchuck killed March 7, 1914, without any anaesthetic. Animal very dormant and had been so nearly all the time for at least three months. Rectal temperature 9°C .

3 After hibernation. Woodchuck killed April 11, 1914, without any anaesthetic. Animal active. Had been fed for two weeks. Rectal temperature 37°C .

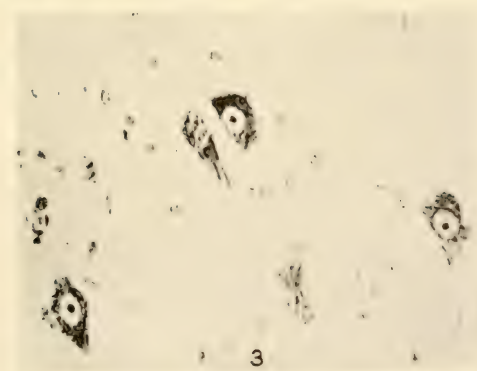
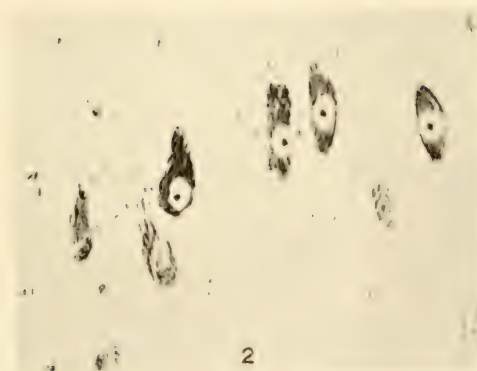
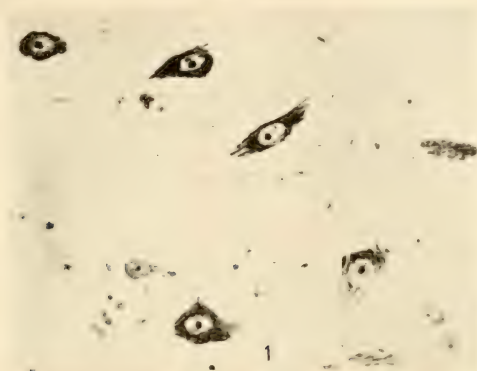
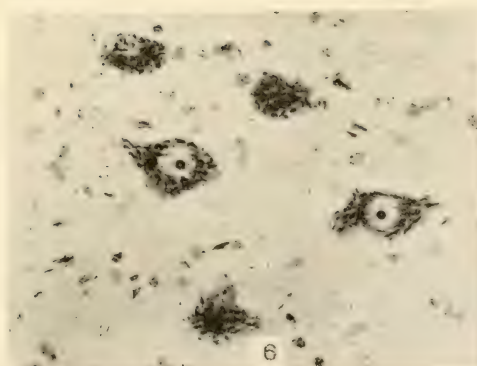
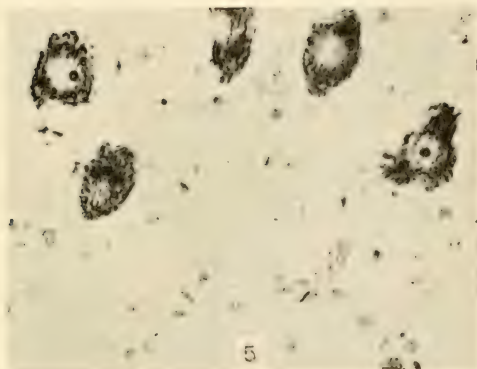
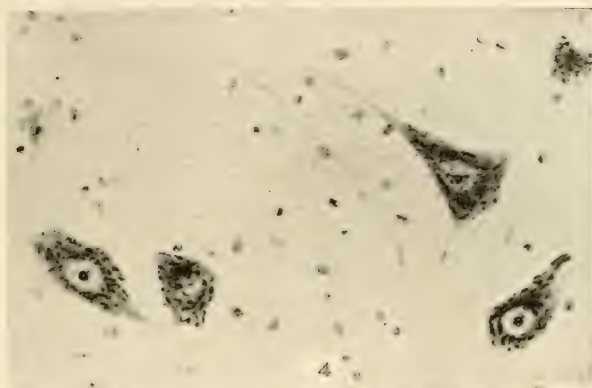


PLATE 2

EXPLANATION OF FIGURES

Photographs of motor cells from the antero-lateral group in the sixth cervical segment of the spinal cord. The sections were mounted on the same slide and hence stained together. $\times 320$.

- 4 Before hibernation. Same animal as in figure 1.
- 5 During hibernation. Same animal as in figure 2.
- 6 After hibernation. Same animal as in figure 3.



A STUDY OF A PLAINS INDIAN BRAIN

J. J. KEEGAN

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EIGHT FIGURES

INTRODUCTION

The primary object of this paper is to furnish an account of the morphology of the cerebral hemispheres of a North American Indian brain, thereby filling a gap in the literature of racial cerebral anatomy. Although a large amount of work has been done upon the Indian tribes from an anthropological standpoint, no description of an Indian brain has, to my knowledge, ever been given. The study of cerebral morphology has not yet furnished characters distinctive of race, but the studies of Elliot Smith ('04), Duckworth ('07), and others have revived an interest in this work and advanced greatly the interpretation of sulci and gyri.

The secondary but more important aim of this study, from which the conclusions are largely deduced, is the application of the principles of fissure relation to cortical areas, both in the interpretation of sulci and in the comparison of cortical areas and sulci of the two hemispheres and of other cerebra. This is especially valuable in this case on account of the completeness of the history and the exceptional mental abilities evidenced.

The study of cortical localization has been conducted by two methods, cortical stimulation and histological differentiation. The most important names connected with the former method are those of Sherrington and Grünbaum ('01) in their experimental work upon the ape brain, supplemented by a few similar observations upon the human cortex and numerous clinical observations proved by operation or autopsy. This work necessarily gave results in only a few regions of the cortex, mainly

the somatic motor and sensory areas of the central region. The histological field was first entered by Flechsig ('96) who delimited cortical areas by the order of the myelination of their fibers. Campbell ('05) and Brodmann ('07) made purely histological surveys of the cortex, but were not able by this tedious method to examine enough material to establish the relation of many cortical areas to fissures. E. Smith ('07) was the first to make such a topographical survey of the human cortex and, while in general the areas plotted agreed with the areas of Campbell and Brodmann, the added value of their interpretation in relation to fissures facilitated greatly the study of the fissures of the cerebrum.

The value of E. Smith's interpretation is evidenced by its general acceptance for the occipital region by most of the recent writers, as Duckworth ('07), Appleton ('10), Cole ('11), Schuster ('08), and many others. The application of this method to other regions, however, has been neglected, chiefly on account of the difficulty in obtaining fresh material and the inexpertness in distinguishing the lamination in macroscopic sections. The boundaries of the area striata are so easily identified that this area can be plotted even in imperfectly preserved material, which accounts for the general application in this region.

In this study an attempt has been made to apply by comparison the cortical area plan of E. Smith ('14) to a valuable cerebrum in which the preservation prevented a knife-section analysis of the cortical areas and their relation to sulci. The accuracy of this method may well be questioned for undoubtedly many small errors are present, but it was found that such a plotting aided greatly in the interpretation of fissures and perhaps made possible a more intelligent comparison of similar cortical areas of the two hemispheres and of other cerebra. It at least furnishes the most expressive figures of the sulci and gyri that present knowledge makes possible and might be applied profitably for comparison to the figures accompanying studies of other brains.

HISTORY

The brain which is the subject of this account was obtained at autopsy from the body of a female Indian of the Omaha tribe, and thanks are due Dr. A. A. Johnson of the Department of Pathology for the opportunity of the use of the specimen for study.

The individual was fifty years of age, about 125 pounds in weight and possessed rather typical Indian features although not extreme. There was united in her person the blood of the Indian, the French, and the English settlers of Nebraska, her grandfathers carrying the half French and the half English admixture and the grandmothers of pure Indian descent. It is thus seen that the Indian blood was carried down entirely on the female side of the family, which might give rise to speculation upon the formerly much discussed question of the relation of sex to cerebral morphology.

The intellectual status was far above the average as superficially judged of Indian people, and above the average of the white race as judged from the high quality of her attainments. The educational training consisted of mission school and government Indian school as a child, five years of general education in preparatory schools, and a three year medical course which she completed in two years, graduating at the head of her class. A year's hospital training completed the medical work. Her life was spent in medical service with her own people. She was generally recognized by the profession and laymen alike as a woman of very superior personality whose intellectual endowments and qualities of character placed her quite above the ordinary level of humanity.

DESCRIPTION

The brain, after standing several weeks in a formaldehyde solution, weighed 1353 grams with the dura mater removed. A comparison of the weights of the two hemispheres showed the right considerably heavier than the left, 605 grams and 575 grams.

respectively, but these figures are unreliable on account of the pathological condition of the left hemisphere, which had destroyed a part of the temporal lobe. This hemisphere also was distorted in preservation, due to the softened condition of the cerebral tissue, consequently no measurements of any value of lengths or indices could be made. Such measurements were established by Cunningham ('92) but have not proven of comparative value even in perfectly preserved brains.

The general type of fissuration presented nothing very unusual, giving the impression of a fairly well and evenly fissured cerebrum. There was no undue prominence of any region, perhaps a greater tendency to a vertical course of the sulci of the central region of the right hemisphere and a tendency to irregular fissuration in the posterior parietal region of the left hemisphere. There was no indication of an exposure of the insula.

The following brief account of the sulci and gyri is not intended as a description of all points observed, for many of these of no recognized significance or variation can be determined equally as well by an examination of the accompanying figures. These illustrations were made by tracing from photographs and subsequently inking in the fissures by careful examination of course, depth and bridging gyri. The depth of the more important fissures is entered in the drawing. In general the heavier lines indicate the deeper fissures. A bridging gyrus is indicated by an interruption of the line and an intervening dot. The cortical areas are filled in with different symbols, the plan being to place no two of similar type adjoining.

These areas were determined by placing those first in which there could be little doubt of their boundaries. The area striata was delimited by macroscopic examination of knife sections. The remaining areas generally could be filled in by a process of elimination, but in some cases they were determined by arbitrary judgment. This latter is very evident in cases where the boundary does not correspond to a fissure, but it is surprising how little is left to the judgment and how easily the area conformation can be made to agree with the plan of E. Smith without violating the rule of the deeper fissures being in the main bounding fis-

tures. With this scheme it is hardly necessary to label the different fissures, for a comparison with E. Smith's figures in Cunningham's Text-book of Anatomy will show the interpretation.

The *fissura cerebri lateralis* is sharply upturned and bifurcated in the left hemisphere at its posterior extremity, but horizontal and single in the right. Since this bifurcation is caused by an overgrowth of the posterior inferior parietal region, as has been determined in the Negro brain by Poynter and Keegan ('15), the two hemispheres are peculiarly contrasted in their centers of greatest growth in the inferior parietal lobule, the left being in the posterior region and the right in the anterior region. This is further evidenced in the conformation of other sulci in these areas, and will be discussed at a later point. In both hemispheres there is a distinct operculum by the superior lip in the region of the area subcentralis. The anterior rami of the fissure are completely separate on both sides, enclosing a prominent *pars triangularis*.

The *sulcus centralis* is unusual in its upper third in the left hemisphere. It lacks several millimeters of reaching the mesial border and in the superior third is displaced anteriorly in an arcuate form, apparently due to an overgrowth of the *gyrus centralis* posterior opposite the motor area of the lower limb. According to Cole ('10), interruption of the sulcus by a submerged or bridging *gyrus* occurs at the posterior arch of the superior genu in poorly convoluted brains and just above in well developed brains. This brain shows the *gyrus* exactly at the apex of the superior genu in both hemispheres, narrower and more prominent in the left. An examination of a large number of Negro and Caucasian brains failed to corroborate Cole's conclusion. The *gyrus* is always at the same point, varying only in depth and width. The maximum depth of the *sulcus centralis* is about the same as the *sulcus interparietalis*. The greater depth of the latter is stated by Appleton ('10) to be a distinctive feature of Australian and simian brains. It is a question whether this would not simply indicate an overgrowth of the parietal lobe in a human brain and not an undergrowth of any region.

The sulci precentrales are typical of Cunningham's ('92) description in the right hemisphere, but in the left hemisphere the superficial interrupting gyrus occurs lower, apparently between the two rami of the sulcus precentralis inferior. The sulcus diagonalis is incorporated in the ramus verticalis in both hemispheres, thus reducing the posterior area of the gyrus frontalis inferior to a small area in the floor of the sulcus precentralis inferior. The factor which would account for this might be an overgrowth of the motor area of the mouth region in the gyrus precentralis inferior or of the motor speech center in the gyrus frontalis inferior. Cole ('10) has attempted to relate the submergence of this area or gyrus to a simian condition and quotes Kohlbrügge and Bolk as supporting his contention that the sulcus diagonalis represents a detached portion of the simian sulcus precentralis inferior.

The sulcus frontalis superior is well defined in both hemispheres and superficially continuous from the s. precentralis superior to the s. fronto-marginalis, maintaining about an even distance from the mesial border throughout. A prominent bridging gyrus near the posterior extremity permits the interpretation of the continuity of the posterior middle frontal region with the anterior superior frontal region as illustrated in E. Smith's chart. Appleton ('10) considers the tendency towards extra segmentation of the s. frontalis superior as a lowly character.

The sulcus fronto-marginalis is better defined in the left hemisphere than in the right but is too irregular in both to permit of much comparison.

The sulcus frontalis inferior is a high arcuate fissure on both sides in full communication with the s. precentralis inferior. Its length and its distance from the fissura cerebri lateralis are each about a centimeter greater in the right hemisphere. This gives rise to a larger gyrus frontalis inferior on the right side which would be contrary to expectation if the speech center had any relation to the size of this gyrus.

The sulcus frontalis medius is a very subordinate system of shallow sulci, according to the interpretation of the s. frontalis superior. The greater width of the gyrus frontalis medius of

the left hemisphere does not seem to increase the regularity of these elements. The greater tendency is towards a transverse direction.

The sulcus frontalis mesialis is seen as an irregular system of alternating transverse and longitudinal elements extending to the frontal pole. The more posterior of these were chosen arbitrarily as a guide between the superior frontal area and the anterior frontal area.

The orbital surface has the usual type of fissuration, the only unusual feature being the extension of the mesial limb of the *s. orbitalis transversus* into the fossa Sylvii of both hemispheres.

The sulcus cinguli corresponding to E. Smith's chart is very poorly developed in its anterior two-thirds, the more prominent sulcus being the *s. paracinguli*. This might indicate a disappearance of the *s. cinguli* due to an increased growth of the mesial region, but was called to attention by Cole (10) in a microcephalic brain in which he suggested that the supracingulate sulcus was the older.

The sulcus postcentralis is a very prominent fissure in both hemispheres, extending from the mesial border to within a few millimeters of the fissura cerebri lateralis. In the right hemisphere there is no communication with the *s. interparietalis*, thus giving rise to a fissure very similar to the *s. centralis*. In the left hemisphere the posterior reflection of the two extremities forms an arcuate sulcus and a wide gyrus centralis posterior in the corresponding regions.

The sulcus interparietalis is contrasted on the two sides. In the right hemisphere it appears as a fourth vertical sulcus in series with the precentral, central and postcentral sulci. It communicates across an almost superficial bridging gyrus with the *s. paroccipitalis*. The tendency in the left hemisphere is towards a sagittal and more posterior arrangement. The sulcus is represented by two horizontal elements in intercommunication with the *s. postcentralis* and the body of the *s. paroccipitalis* across bridging gyri.

The sulcus paroccipitalis is more lateral and more prominent in the right hemisphere. The independence of this sulcus is

well represented in both hemispheres. Appleton ('10) has classified this as a fetal tendency. The narrow antero-posterior width of the gyrus arcuatus posterior is a noticeable feature and might be indicative of an unusual growth in the superior parietal area.

The sulcus parietalis superior is poorly represented, perhaps due to its partial incorporation in surrounding sulci.

The lobulus parietalis inferior gives evidence of a prominent growth. This is more noticeable in the left hemisphere where the prominent sulcus interparietalis extends into the anterior region as an accessory fissure. The s. angularis, which to some extent is an index to the growth of the inferior part of this lobule, is not independent from the s. temporalis superior as found in the majority of Negro brains. It appears as the sharply upturned extremity of this fissure, more anterior in the left hemisphere.

The sulcus temporalis superior of the right hemisphere was injured by the tumor growth. The absence of the anterior transverse element may be associated with an increased growth of the acoustic area of the superior temporal gyrus. This was further evidenced by very prominent transverse temporal gyri of Heschl and the extension to the lateral surface of the fissure separating the two larger of these gyri.

The sulcus temporalis medius and the sulcus temporalis inferior have never been interpreted well enough to permit morphological comparison. The ascending ramus of the former, sulcus occipitalis anterior of some authors, is typical in the right hemisphere of an arcuate communication with the s. occipitalis inferior, classified by Appleton ('10) as a simian character.

The fissura rhinalis is present in both hemispheres as a shallow groove connecting the Sylvian fossa with the s. collateralis. The interpretation of these two fissures, applied as in the Negro brain, indicates an earlier or less developed condition in the right hemisphere but in neither is the simian or fetal type approached, regardless of the greater depth than usual.

The sulcus collateralis is superficially continuous to the occipital region in both hemispheres. The posterior portion lies considerably more lateral in the right, which, as a limiting sulcus for the area peristriata, would indicate a greater extent of this

area on this side. The rhinal element of the sulcus is separated in both hemispheres by a bridging gyrus near the anterior extremity.

The sulcus lunatus can be very plainly identified in the left hemisphere. It is 35 millimeters in length and lies nearer the lateral border about 3 centimeters from the occipital pole. It has an arcuate or rather angular form and a slightly operculated posterior lip. The area striata, delimited by knife sections, lacked about 2 millimeters of reaching this lip but followed the course of the fissure quite regularly. In the right hemisphere the tendency is towards a longitudinal disposition of the fissures in this region, to which the boundary of the area striata does not bear any definite relation. The most prominent sulcus is interpreted as a modified *s. prelunatus* or *s. occipitalis lateralis* of some authors. Comparison of the extent of the area striata in the two hemispheres shows very plainly a greater lateral extent in the left hemisphere.

The sulcus occipitalis paramesialis lies more upon the lateral surface in the left hemisphere but is very prominent in both hemispheres. This lateral position indicates that it bears a relation to the lateral extension of the area striata.

The sulcus occipitalis inferior courses on the tentorial surface along the lateral border. It is very similar on the two sides, a deep, slightly operculated fissure about 40 millimeters in length, communicating at the two extremities with the sulci of the corresponding regions.

The sulcus calcarinus in both hemispheres is separated from the fossa parieto-occipitalis by a small bridging gyrus cuneus and from the sulcus retrocalcarinus by a larger bridging gyrus.

The sulcus retrocalcarinus terminates in both hemispheres in a deep operculated polar element but separated by a prominent bridging gyrus. Landau ('15) interprets this polar element (*s. extremus*, *s. occipitalis triradiatus*, *s. calcarinus externus* of E. Smith, *s. occipitalis polaris*), as the true posterior bifurcation as described by Cunningham ('92), separated by the gyrus cuneo-lingualis posterior and homologous to the sulcus triradiatus of ape brains. The prominence of this sulcus was noted by the writer ('15) in the Negro brain, in which it was described as an

independent sulcus occipitalis polaris. If the interpretation of Landau is correct, its prominence would be an indication of a greater proportionate development of the area striata in the same manner as the sulcus lunatus.

The fossa parieto-occipitalis is quite typical of E. Smith's description in the left hemisphere. The gyrus intercuneatus is about 5 mm. in height and separates widely the s. paracalcarinus and the s. limitans praecunei. It is traversed by an independent sulcus incisura which appears superficially on the lateral surface, separate from the termination of the sulcus limitans praecunei in the gyrus arcuatus posterior. The s. paracalcarinus undermines the posterior wall of the fossa, becoming superficial along the border of the hemisphere posterior to the gyrus arcuatus. The fossa of the right hemisphere is less typical. The gyrus intercuneatus is faintly indicated, the deepest part of the incisura being formed by the sulcus paracalcarinus. The s. limitans praecunei incises the lobulus praecuenus.

The sulci limitantes areae striatae are easily identified by the delimitation of the area striata.

The insula, as far as could be determined, presented nothing unusual in its fissuration. The operculum was complete in both hemispheres.

SUMMARY

The detailed study of the fissures and convolutions brings a number of points to attention. First is noted the striking difference between the two hemispheres in almost every fissure or region examined. This variation is so great that a comparison of hemispheres is of very little value in the interpretation of sulci, which method proved so valuable in the interpretation in the Negro brain. A similar fact of asymmetry is stated by Appleton ('10) to represent an agreement with European cerebra in general and a contrast with supposedly lower types of cerebra. Many writers have called attention to the asymmetry of the cranium, brain, and intracranial venous sinuses separately, but comparatively little work has yet been done to study the co-relation of these asymmetrical conditions the one to the other or to explain their origin. E. Smith ('07), in a note upon

the asymmetry of the brain and skull, contrasts the asymmetry of the cranium of the higher races of man with the symmetry of the apes and to a less degree of the black races. This lack of symmetry of the cranium in man is attributed to the unequal development of homologous parts of the two cerebral hemispheres, especially the great parietal and frontal association areas. The greater size of the right parietal association area is given as an explanation of the greater prominence of the corresponding parietal bone and a relative shifting backward of the right parietal boss, of the usually greater extent of the lateral part of the left visual cortex and the retention of a more pithecoïd form in the left hemisphere.

This asymmetry is suggestive of the predominance of functional areas upon one side or the other of the brain. This has been generally accepted in the predominance of the speech center upon the left side in the inferior frontal gyrus, Broca's convolution, and of the visual cortex in the more extensive area striata and more prominent sulcus lunatus in the left hemisphere of the majority of brains. Cunningham ('02) attributed right-handedness to a functional pre-eminence of the left hemisphere, although not supported by any constant observation of greater weight or greater prominence of the so-called motor arm center, of appreciable histological difference or of any regular plan of asymmetry. The asymmetry, although noticeable also in the lower animals, "never attains the same degree as in man." A deficient growth of the ascending parietal convolution (gyrus post-centralis) has been found associated with the congenital absence of the arm of the opposite side (Moorhead, '02). The fact that this is not in the so-called motor center of the arm is interesting in that it shows that the functional center does not necessarily correspond to the motor center.

The importance of this aspect of cerebral morphology would tend to complicate the comparison of sulci and gyri, not only between the two hemispheres of the one cerebrum but also between hemispheres of different cerebra, of individuals or of races. It would be necessary, before a rational comparison could be made, to establish as many points as possible of functional similarity. At the present time there are few such points for

comparison, but it is not unreasonable to expect that a closer clinical or functional analysis may disclose more features that can be translated as indications of the predominance of functions upon one side or the other of the brain. An interesting observation would be to determine if a predominant vision with the right eye is related to a more prominent area striata and sulcus lunatus of the left hemisphere.

The most striking differences between the two hemispheres of this brain are found in the occipital, parietal and central regions. The sulcus lunatus can be identified only in the left hemisphere where it is very prominent and associated with a more lateral extent of the area striata, a larger, more lateral and more independent sulcus occipitalis triradiatus, a sulcus occipitalis paramesialis more upon the lateral surface, a sulcus occipitalis inferior nearer the lateral surface and in arcuate communication with the sulcus occipitalis anterior.

The inferior parietal area is more extensive in the right hemisphere and the increased growth appears to be in the anterior portion. This is evidenced by the extension of the anterior extremity of the sulcus interparietalis into this region and the more posteriorly situated sulcus angularis. The conformation of the sulcus interparietalis of the left hemisphere would seem to indicate a predominance of growth in the superior parietal lobule. This has resulted in a low position of the sulcus interparietalis, a posterior deflection of the sulcus post-centralis and a striking anterior arching of the sulcus centralis above its superior genu. The lower part of the gyrus post-centralis is also widened by the posterior deflection of the inferior extremity of the sulcus post-centralis. The entire gyrus is noticeably wider than in the right hemisphere.

The value of these observations of the dissimilarity of the two hemispheres is difficult to judge on account of the lack of knowledge of the extent of unilateral functional predominance and of clinical tests to corroborate the morphological findings. The study is interesting from an anthropological standpoint, demonstrating that in a race of inferior status all of the elements necessary for a higher individual development are present. The same condition was concluded in the Negro brain (Poynter

and Keegan, '15). No single morphological point could be selected which would represent inferiority. While in the Negro the mental characteristics may be in part explained by the great predominance of the parietal lobe over the frontal lobe, in this brain the characteristic feature is not a disproportionate growth of any large area, nor any striking complexity or simplicity of fissuration, but a marked asymmetry of the fissures and convolutions.

CONCLUSIONS

1. This Indian brain represents in practically all features a high type of cerebrum, the only possible exceptions being the shallow communication of the incisura rhinalis with the sulcus collateralis and the presence of a typical sulcus lunatus in the left hemisphere.

2. The great asymmetry of the two hemispheres in fissuration is the most convincing evidence of a highly specialized cerebrum. Future combination of physiological observation with cerebral morphology will undoubtedly lead to a better interpretation of such variations.

3. The comparison of cerebra of different individuals should take into consideration this relation of asymmetry to functional localization and should be confined to hemispheres of the same side in individuals of as near similar mental traits as possible.

4. The method of plotting the cortical areas established by E. Smith, although inaccurate in many details, aids greatly in the interpretation of fissures and in the comparison of the development of different areas. It serves as the most expressive manner in which the morphology of cerebra can be presented for comparison.

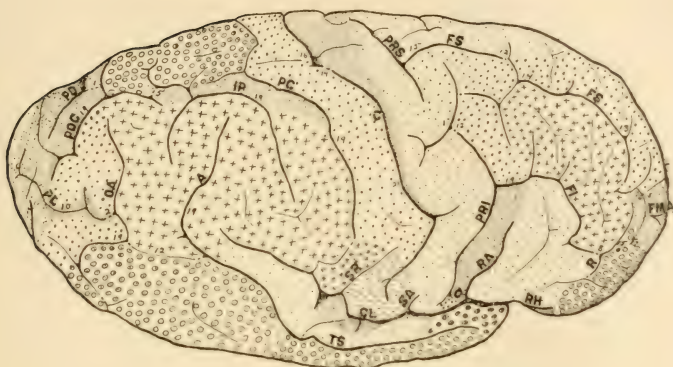
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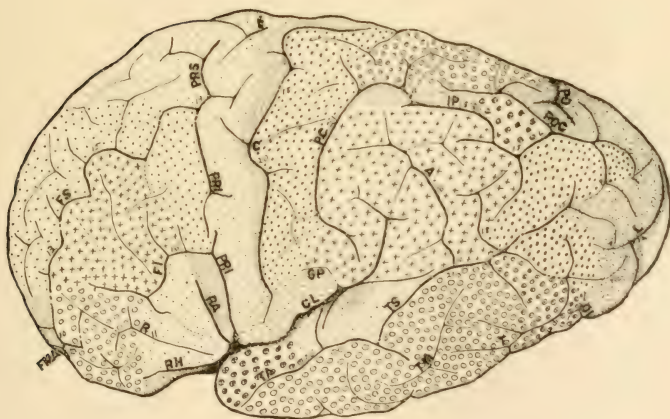
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ABBREVIATIONS

<i>A</i> , Sulcus angularis	<i>PL</i> , Sulcus prelunatus
<i>C</i> , Sulcus centralis	<i>PO</i> , Fossa parieto-occipitalis
<i>CA</i> , Sulcus calcarinus	<i>POC</i> , Sulcus paroccipitalis
<i>CL</i> , Fissura cerebri lateralis	<i>PRI</i> , Sulcus precentralis inferior
<i>CO</i> , Sulcus collateralis	<i>PRS</i> , Sulcus precentralis superior
<i>D</i> , Sulcus diagonalis	<i>R</i> , Sulcus radiatus
<i>FI</i> , Sulcus frontalis inferior	<i>RA</i> , Ramus ascendens of fissura cerebri lateralis
<i>FM</i> , Sulcus frontalis medius	<i>RC</i> , Sulcus retrocalcarinus
<i>FMA</i> , Sulcus fronto-marginalis	<i>RH</i> , Ramus horizontalis of fissura cerebri lateralis
<i>FS</i> , Sulcus frontalis superior	<i>RI</i> , Fissura rhinalis
<i>IP</i> , Sulcus interparietalis	<i>RO</i> , Sulcus rostralis
<i>L</i> , Sulcus lunatus	<i>SA</i> , Sulcus subcentralis anterior
<i>OA</i> , Sulcus occipitalis anterior	<i>SC</i> , Sulcus cinguli
<i>OI</i> , Sulcus occipitalis inferior	<i>SP</i> , Sulcus subcentralis posterior
<i>OL</i> , Sulcus olfactorius	<i>TA</i> , Sulcus temporalis anterior
<i>OP</i> , Sulcus occipitalis paramesialis	<i>TI</i> , Sulcus temporalis inferior
<i>OR</i> , Sulcus orbitalis transversus	<i>TM</i> , Sulcus temporalis medius
<i>P</i> , Sulcus occipitalis polaris (retro-calcarine bifurcation)	<i>TS</i> , Sulcus temporalis superior
<i>PC</i> , Sulcus postcentralis	
<i>PCI</i> , Sulcus paracinguli	



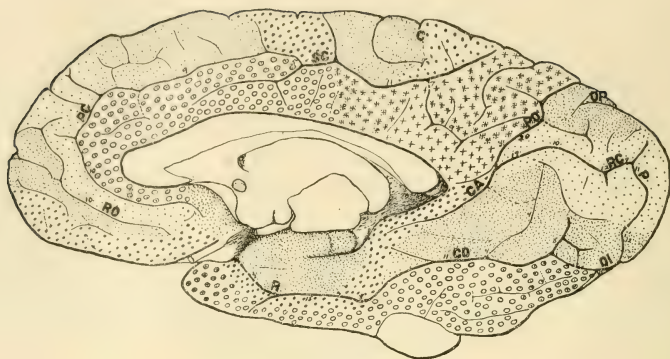
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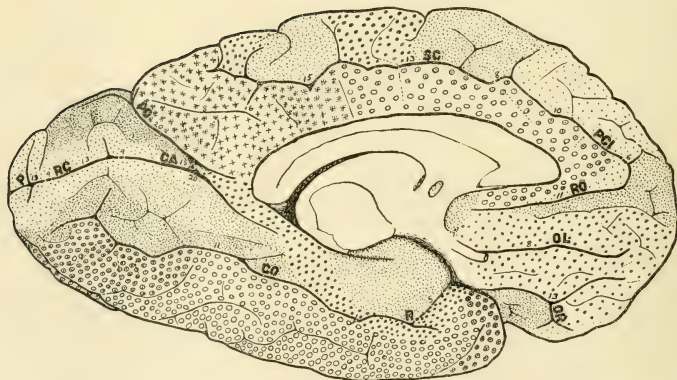
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Fig. 1 Right hemisphere, lateral view

Fig. 2 Left hemisphere, lateral view



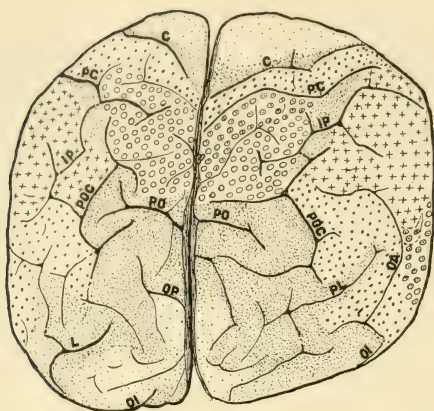
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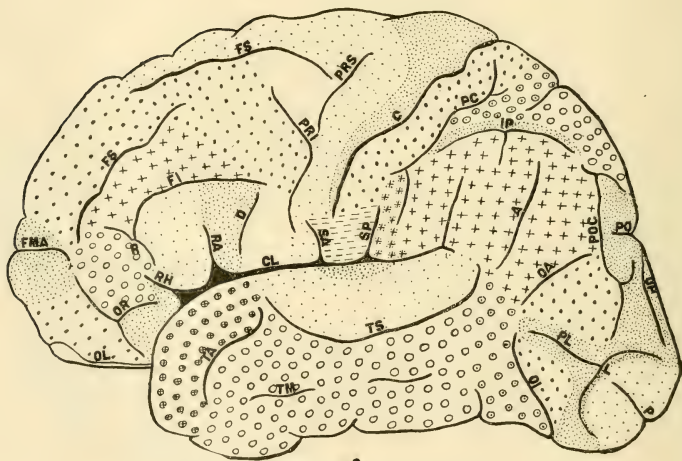
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Fig. 3 Right hemisphere, mesial view

Fig. 4 Left hemisphere, mesial view



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8

Fig. 7 Both hemispheres, occipital view

Fig. 8 Copy of E. Smith's ('14) figure

AN EXPERIMENTAL STUDY OF THE VAGUS NERVE

MARTIN R. CHASE

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FOUR FIGURES

In a previous paper a study was made of the structure of the roots, trunk and branches of the vagus nerve (Chase and Ranson '14).

It was shown, in conformity with the work of Molhant ('10) and Van Gehuchten and Molhant ('11) that the vagus nerve in the dog contains myelinated fibers which can be classified as large, medium and small. In addition there were found enormous numbers of unmyelinated fibers. These are present in certain of the vagus rootlets, and throughout the trunk of the vagus. In the cervical and thoracic trunk they constantly increase in proportion to the myelinated fibers, so that at the level of the diaphragm the vagus is almost a pure unmyelinated nerve.

The most of the large myelinated fibers, and many of medium size, are given off in the cervical branches of the vagus. The bronchial and esophageal rami receive nearly all of the remaining myelinated fibers. Relatively few unmyelinated fibers are found in the cervical branches. The increased proportion of unmyelinated fibers in the lower vagus is due in part at least to the withdrawal from the trunk of the myelinated fibers by the upper branches. Essentially the same histological picture was seen in sections from the vagus of man (Ranson '14), the rat and the rabbit.

Gaskell '86 observed unmyelinated fibers in the vagus nerve, and found the lower thoracic vagus to contain few myelinated fibers. He interpreted the unmyelinated fibers in the vagus

¹ Contribution No. 37.

as being post-ganglionic fibers arising from sympathetic cells in the vagus ganglia.

Molhant '10 working with the Cajal reduced silver method, saw masses of unmyelinated fibers in the vagus nerve and thought they were of sympathetic origin, being presumably post-ganglionic fibers arising from cells in the sympathetic ganglia. Neither Gaskell nor Molhant demonstrated unmyelinated fibers in the roots of the vagus.

Langley has suggested the possibility of preganglionic visceral efferent fibers losing their myelin sheaths during their course down the vagus.

Ranson ('15) has recently studied the structure of the vagus nerve in the turtle, and has made observations of importance in clearing up the origin of the unmyelinated fibers in the vagus nerve. The vagus nerve in the turtle divides high in the neck into a cervical and a thoraco-abdominal ramus. The cervical ramus is composed almost entirely of myelinated fibers and the cells of the cervical ganglion of the vagus are associated only with the fibers of this ramus.

The thoraco-abdominal ramus presents the same structure as the thoracic vagus in mammals. It has a ganglion in the upper part of the thoraco-abdominal cavity. It consists largely of unmyelinated fibers, with scattered myelinated fibers, and maintains the same structure from its origin to the origin of the recurrent nerve. Ranson shows that the unmyelinated fibers are not post-ganglionic fibers arising from cells in the cervical ganglion, since the thoraco-abdominal trunk is not associated with this ganglion. Neither do preganglionic visceral efferent fibers lose their myelin sheaths, since the structure of the thoraco-abdominal ramus does not change. There is no association with sympathetic ganglia, and the proportion of unmyelinated fibers does not change below the thoraco-abdominal ganglion, so there are probably no sympathetic elements in this ganglion.

In the dog there is a very close association of the vagus and sympathetic trunks in the neck, and it seemed possible that some of the unmyelinated fibers of the thoracic vagus in this animal

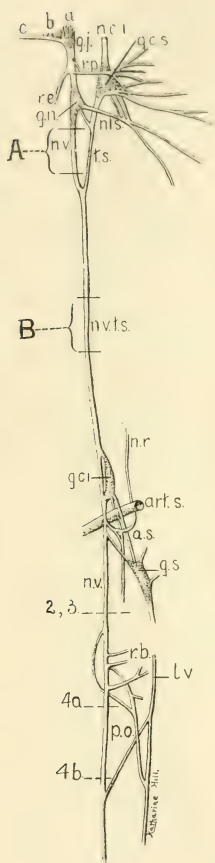
might be of sympathetic origin. The present paper presents the results of some experiments performed on dogs to determine by degeneration methods to what extent sympathetic fibers entered into the composition of the vagus.

Figure 1 is a diagram of the right vagus nerve in the dog, showing the relations with the sympathetic, and the origin of the branches. Note that the two trunks are closely united from high in the neck to the upper thoracic region. Note also that just below the bronchial rami, *r.b.*, each vagus gives off a branch which goes to unite with the nerve of the opposite side. In addition each nerve is also joined by smaller twigs from the contra-lateral vagus. Sections of either vagus below the level of the bronchial rami may include fibers which originate in the opposite vagus, a fact of importance in interpreting the histology of the degenerated nerves.

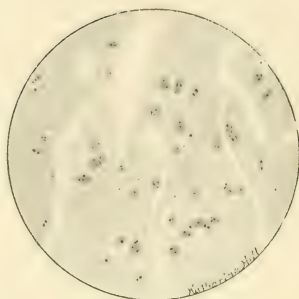
It would be possible for post-ganglionic fibers to enter the vagus from communications with the superior cervical ganglion of the sympathetic. In the preparation of the former paper (Chase and Ranson) we were unable, in a study of serial sections, to trace any considerable number of such fibers into the vagus. Throughout their course in the neck, although the two nerves are contained in a common sheath, no interchange of fibers could be traced. At the level of the inferior cervical (*g.c.i.*) ganglion of the sympathetic, however, the association of the vagus and sympathetic is very close, and numerous large bundles of sympathetic unmyelinated fibers can be seen in serial sections entering and leaving the vagus trunk. If, then, any considerable proportion of the unmyelinated fibers in the thoracic vagus of the dog have their origin in the sympathetic trunk, they must arise from cells in the inferior cervical ganglion *g.c.i.*, or ganglion stellatum, *g.s.*

METHODS

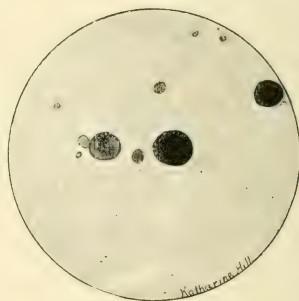
The right vagus nerve was severed in a number of dogs. In some cases it was separated from the sympathetic trunk high in the neck and about an inch of the vagus nerve was removed (fig. 1, A). There would then be no interference with fibers



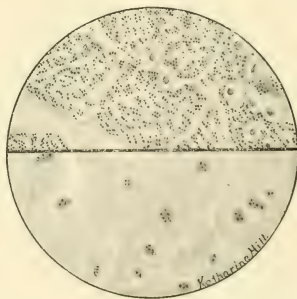
1



2



3



4 a.

4 b.

from the superior cervical ganglion, the sympathetic trunk or inferior cervical ganglion.

In other cases about an inch of combined vagus and sympathetic trunk was removed in the midcervical region (fig. 1, *B*). Such a procedure would cut off any fibers to the vagus from the superior cervical ganglion, but would in no way interfere with fibers from the inferior ganglion.

The dogs were autopsied after 2 to 8 weeks and portions of the nerves studied after preparation by the pyridine silver method (Ranson '11, '12) and the osmic acid method.

CHANGES AFTER SECTION OF THE VAGUS ALONE HIGH IN THE NECK

Sections from the right vagus of a dog autopsied five weeks after removal of a portion of the upper cervical vagus, the sympathetic being left intact, show definite degenerative changes. A cross section stained by the pyridine silver method, taken

Fig. 1 Diagram of the right vagus nerve in the dog

Fig. 2 From a section of right vagus nerve at level indicated (fig. 1, *2, 3*), following removal of a portion of the vagus and sympathetic trunks at level *B*, figure 1. Pyridine silver. $\times 1140$.

Fig. 3 From a section of the same nerve at same level as figure 2. Osmic acid. $\times 1140$.

Fig. 4a From a section of a normal vagus nerve just below pulmonary plexuses (fig. 1, *4a*). Pyridine silver. $\times 1140$.

Fig. 4b From a section of the same nerve as figure 2, below the pulmonary plexuses (fig. 1, *4b*). Pyridine silver. $\times 1140$.

a, vagus rootlets

A, B., indicate levels at which portions of the vagus trunk were removed

art.s., arteria subclavia

a.s., ansa subclavia

b, bulbar rootlets of the accessory nerve

c, spinal root of the accessory nerve

g.c.i., ganglion cervicale inferior

g.c.s., ganglion cervicale superius

g.j., ganglion jugulare

g.n., ganglion nodosum

g.s., ganglion stellatum

l.v., left vagus

n.c.i., nervus caroticus internus

n.l.s., nervus laryngeus superior

n.r., nervus recurrens

n.v., nervus vagus

n.v.t.s., nervus vagus and truncus sympathicus

p.o., plexus esophageus

r.b., rami bronchiales

r.e., ramus externus n. accessorii

r.p., ramus pharyngeus

t.s., truncus sympathicus

2, 3, 4a, 4b, indicate levels at which the corresponding figures were taken

through the thoracic vagus just above the bronchial rami, shows no normal myelinated fibers and only a few scattered unmyelinated axones. There are present, however, a few bundles of unmyelinated fibers which are clearly of sympathetic origin. They cling in groups and stain in a characteristic manner. Similar groups of unmyelinated fibers, present at this level in the normal vagus nerve, were readily identified as of sympathetic origin by their contrast with the vagus fibers. They occupy only a small part of the total cross section area.

Sections taken below the pulmonary plexuses show the right vagus to have retained its degenerated character. It is joined early by twigs from the normal left vagus, whose deeply stained fibers contrast sharply with the degenerated areas. It is impossible to locate in these sections the sympathetic fibers seen at a higher level. They are not present in bundles, and it would be impossible to differentiate individual sympathetic fibers from normal fibers from the left vagus.

CHANGES PRODUCED BY REMOVAL OF A PORTION OF THE CERVICAL VAGO-SYMPATHETIC TRUNK

In the animal from which the illustrations are taken, a portion of the right sympathetic vagus trunk was removed as indicated (fig. 1, *B*) and the dog was allowed to live four weeks.

Figure 2 is a drawing from a cross section of the right vagus trunk just proximal to the bronchial rami (fig. 1, 2, 3) stained by the pyridine silver method. There are seen no normal myelinated or unmyelinated nerve fibers. The aggregations of granules, surrounded by definite zones, are nuclei of neurilemma cells, as is proven by longitudinal section. The entire cross section shows the same structure, there being only an occasional naked axone. There are no bundles of unmyelinated sympathetic fibers as was seen at the same level in the specimen previously described.

Figure 3 is a drawing of a small part of a cross section of the same nerve at about the same level, stained with osmic acid. Note the presence of a number of deeply stained globules, which are the remains of degenerated myelin sheaths. There is pres-

ent in the entire cross section only an occasional myelin ring, and most of these clearly represent degenerating fibers.

Figure 4 is a composite drawing. Figure 4a, the upper half of the figure, is taken from a cross section of a normal right vagus nerve just below the origin of the bronchial rami (fig. 1, 4a). There are present in this field nine small myelinated axones. The remainder of the field is packed with unmyelinated axones. Figure 4a may be taken to represent the condition in the normal thoracic vagus, it being remembered that above the bronchial rami there are more myelinated axones, while near the diaphragm there are even fewer than in the figure. Figure 4b is a drawing from a cross section of the same nerve as figures 2 and 3, taken at a level just proximal to the union with the branch from the left vagus nerve (fig. 1, 4b). There are in the field no normal unmyelinated or myelinated axones, and the entire cross section shows the same structure, there being only an occasional naked axone.

In both pyridine silver and osmic acid preparations of the nerve below the level described sections, both of the right nerve after its union with the branch from the left vagus, and of the left nerve after union with the branch from the right, show a mingling of the degenerated nerve with the normal, but the degeneration can be clearly traced to the diaphragm.

In the second case, where both the vagus and sympathetic were cut in the mid-cervical region, no bundles of fibers of sympathetic origin could be found, the entire nerve being degenerated.

We conclude, then, that following section of the cervical vagus nerve in the dog, with or without section of the sympathetic trunk, all fibers of the vagus, myelinated and unmyelinated alike, undergo complete degeneration. There are to be found in the degenerated thoracic trunk, above the level of the pulmonary plexuses, in some individuals bundles of unmyelinated nerve fibers having their origin in the cells of the sympathetic, presumably in the inferior cervical ganglion. In some individuals no bundles of this sort are found. In no case does the total number of fibers of sympathetic origin present amount to more than an insignificant fraction of the total number of unmyelinated

fibers normally present. In the material studied it was possible to demonstrate more groups of unmyelinated fibers of sympathetic origin in the thoracic vagus in the animals in which only the vagus was severed high in the neck, than in those operated by division of both vagus and sympathetic trunks. It is thought that this finding is the result of individual variation in the animals, but it is possible that the difference in operative procedure has something to do with it.

The unmyelinated character of the thoracic vagus nerve in the dog is not due to the presence of fibers derived from the sympathetic trunk, during the close association of the vagus and sympathetic nerves in the neck.

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CHANGES IN THE ROD-VISUAL CELLS OF THE FROG DUE TO THE ACTION OF LIGHT

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TWO FIGURES

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PRELIMINARY

Whenever the results of experimentation upon any particular animal, or group of animals, are at variance with a well established canon, it is desirable that such results be subjected to the closest scrutiny possible before the existence of a deviation from the generally accepted norm is admitted. The present paper, which attempts to answer the following query, is concerned chiefly with the reëxamination of a case of this kind—Query: *Are the photomechanical responses of the visual rods of the frog similar to those occurring in the retinas of other investigated vertebrates?*

Both the rods and the cones of many vertebrates undergo positional changes when subjected to photic or thermal stimulation (Arey '15, '16).

¹ Contribution No. 38, April 20, 1916.

Wherever movements of the cones have been detected, light causes a shortening, and darkness an elongation, of the contractile portion of the inner member known as the myoid (figs. 1 and 2, *my.con.*).

Stort ('86), working on the crow, first showed that the rod myoid of birds exhibits photomechanical changes which are the exact reverse of those found in the cone—that is, elongation in the light and retraction in the dark. The following year ('87) he extended his observations upon certain fishes. Although this striking response in fishes can be demonstrated with comparative ease (especially in fishes possessing large rods; Arey, '15, '16), it was not until long after that these forgotten observations were corroborated and their correctness admitted.

With respect to the frog's rod,² however, Angelucci ('84, '90) and Gradenigro ('85) came to a different conclusion. These workers recorded that the myoid of the inner member (figs. 1 and 2, *my.bac.rb.*) of the frog's rod shortens when exposed to light and elongates in darkness. Gradenigro (p. 343) states this conviction most emphatically: "Zuerst habe ich mit positiver Bestimmtheit ersehen dass an der Froschretina unter der Einwirkung des Lichtes die Stäbcheninnenglieder kürzer und dicker werden, in ähnlicher, wenn auch nicht so ausgedehnter und rascher Weise, wie Englemann bei den Zapfennengliedern die Beobachtung gemacht hat."

Angelucci ('90, p. 245) makes a similar straightforward assertion, which applies to both the red and green visual rod:

² There are two kinds of visual rods in the frog's retina. The more numerous form (figs. 1 and 2) has a short inner member (*my.bac.rb.* + *ell.bac.rb.*) and a long outer member (*prs.dst.bac.rb.*). Since the first imperfect observation by Müller ('51), it has been shown repeatedly that, in the fresh retina of an animal which has previously been retained in darkness, the outer member of the rod appears reddish due to the presence of unbleached visual purple (rhodopsin). Schwalbe ('74) saw a second type of rod (figs. 1 and 2) characterized by an elongated inner member (*my.bac.vr.* + *ell.bac.vr.*) and an abbreviated outer member (*prs.dst.bac.vr.*); Boll ('77) also described this element and interpreted correctly its green appearance, in fresh dark-adapted retinas, as due to a specific photo-sensitive material, which, from its color has been called visual green (chloianopsin). Hence red and green rods differ both anatomically and as to the nature of their photo-sensitive contents. Unless otherwise stated reference to the red visual rod is usually understood.

"Die Stäbchen sind dick und sowohl in ihrem äusseren Glied als in ihrem myoïden Theil zusammengezogen. . . . Auch die grünen *Schwalbe*'schen Stäbchen sind sowohl in ihrem äusseren als in ihrem inneren Gliede zusammengezogen."

Arcoleo ('90), using pithed toads, likewise reported similar responses.

More recently, Lederer ('08), in a brief communication, has challenged the results of the previously named workers on the frog's rod. From the study of fixed material, which had been subjected to teasing, he concludes (p. 764): "Die Hellstäbchen waren im allgemeinen länger, schlanker und hatten gleich breites Innen- und Aussenglied, während bei den Dunkelstäbchen, die kürzer und plumper erschienen, das Innenglied dort, wo es an das Aussenglied grenzt dicker wird." It should be remarked in passing that Lederer's two schematic figures of isolated frog's rods have presumably been interchanged—at least, it is obvious that they illustrate conditions exactly the reverse of those which his text descriptions maintain. His experience with stained celloidin sections is summarized in the following statement (p. 764):

... man nach den Zupfpräparaten am gefärbten Schnitte ebenfalls ähnliche Verhältnisse hätte erwarten sollen. Indessen waren hier die Veränderungen der Stäbchenschicht sehr wenig markant. In ungefähr der Hälfte der geschnittenen Licht-Bulbi zeigten die Stäbchen längere, gestrecktere Form, grösseren Abstand ihres Aussengliedes von der *Membrana limitans externa*, die Dunkelstäbchen gedrungenere gestalt, kleinere Distanz von der äusseren Grenzmembran. In der anderen Hälfte der Schnitte aber war eine Veränderung der Stellung der Stäbchen sehr wenig ausgesprochen, und die Hell- und Dunkelstäbchen das Aussenglied ungefähr gleich weit von der *Membrana limitans externa* entfernt.

The rods of certain amphibians exhibit photomechanical movements which should be clearly distinguished from those produced by the contractility of the myoid. Thus Stort ('87) first asserted, that, in the dark, the nuclei of the rods in Triton (figs. 1 and 2, *st.n.l.cr.*) migrate partially through the external limiting membrane, thereby causing the whole rod to become extended, whereas, in the light, these nuclei lie wholly within the outer nuclear layer. The contractility of that portion of the

rod-visual cell between the rod nucleus and the external reticular layer was believed to cause these changes. Angelucci ('90) made similar observations on the salamander, as did Garten ('07) on Triton.

Changes in the cylindrical outer member (figs. 1 and 2, *prs.dst.* *bac.rb.*) have also been reported. Ewald und Kühne ('78) first observed a swelling of the outer member of the frog's rod as the result of strong illumination. That Lederer ('08) obtained

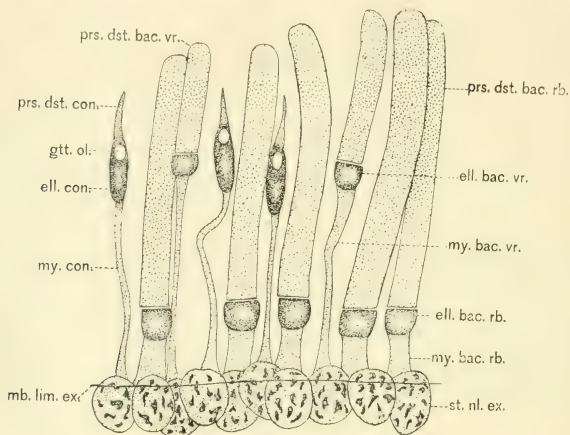


Fig. 1 From a dark-adapted retina of the frog, *Rana pipiens*, showing the positions assumed by the rods and cones. $\times 1130$. *ell.bac.rb.*, ellipsoid of red rod; *ell.bac.vr.*, ellipsoid of green rod; *ell.con.*, cone ellipsoid; *gtt.ol.*, oil globule; *mb.lim.ex.*, externa limiting membrane; *my.bac.rb.*, myoid of red rod; *my.bac.vr.*, myoid of green rod; *my.con.*, cone myoid; *prs.dst.bac.rb.*, outer member of red rod; *prs.dst.bac.vr.*, outer member of green rod; *prs.dst.con.*, outer member of cone; *st.nl.ex.* external nuclear layer.

results, the exact opposite of those reported by Ewald and Kühne, is assumed from the context of the previously cited quotation (p. 431), and from his illustrations.

Angelucci ('84) measured the length of the outer member of the frog's rod and found it shortens in the light; later ('90) he confirmed this result by measurements of the large rods of the

salamander where the differences in length were more striking. Arcoleo ('90) and Garten ('07) reported similar conditions for the toad and frog respectively.

In most accounts of the changes occurring in the anuran rod, due to the action of light, reference is not made to the number of individuals experimented upon, and in no case are definite measurements of length given, judgment of the eye apparently being the only criterion adopted.

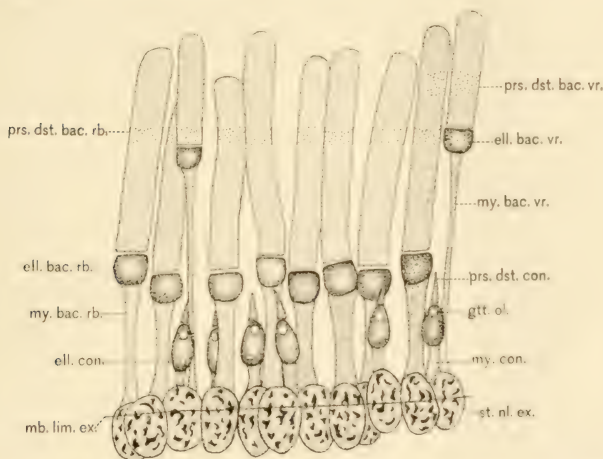


Fig. 2 From a light-adapted retina of the frog, *Rana pipiens*, showing the positions assumed by the rods and cones. $\times 1130$.

In the experimentation which forms the basis of this paper, an attempt has been made to correlate an influence of light with the following possible changes in the frog's visual rod: 1) changes in the position of the red rod nucleus with respect to the external limiting membrane; 2) changes in the length of the red and green rod myoids; 3) relative changes in the length of the red rod myoid at the center and at the periphery of the retina; 4) changes in the diameter of the various component parts of the red rod.

EXPERIMENTATION

Individuals of *Rana pipiens*, approximately uniform in size, were exposed to bright diffuse daylight for eight hours, or to total darkness for a minimum length of twenty-eight hours. At the expiration of these periods of light and dark-adaption, the cranium of each animal was first split sagittally, and then cut transversely just caudad to the eyes. The resulting moieties of the cranium, with the contained eyes, were dropped into Perenyi's chromo-nitric fixative; in this solution they underwent fixation, the condition of illumination being identical with that to which they had been experimentally subjected. The operation on dark-adapted animals was accomplished by the light from a photographic red lamp and demanded but a few seconds time.

After dehydration and the removal of the lens, the eyes were imbedded in paraffine, sectioned 8μ thick, and stained with Ehrlich-Biondi's acid fuchsin-orange G-methyl green mixture. In light-adapted retinas the expanded pigment masked the visual cells to a greater or less degree, hence, nascent oxygen was used as a bleaching agent in these preparations. The results obtained with the Ehrlich-Biondi stain were very satisfactory, yet it is of interest to note that the staining reaction of several definite structures was often variable. For example, the outer member of the red rod in most cases stained red with the acid fuchsin, yet in some retinas from the same series they were colored an intense orange from the orange G, although the treatment in both cases had been, so far as is known, identical, the series having been carried along simultaneously step by step. Moreover, in one preparation, at least, it was observed that the outer members in one half of the retina were stained orange, whereas in the other half they were stained red. This selective stainability is doubtless indicative of a variable physiological cytoplasmic state. Preparations in which the outer members of red rods selected the orange G, showed the green rods stained red with the acid fuchsin. In retinas that had been bleached (potassium chlorate and hydrochloric acid being the reagents

used), the rod outer member varied in color from blue-violet to red-violet. Cases involving a somewhat similar variability might be cited with respect to several other structures in the retina.

Experimentation was made upon sixty retinas, from which twenty-three light-adapted and twenty-three dark-adapted preparations were selected for measurement. Preparations, in which the external limiting membrane was not apparent, or in which wrinkles caused oblique sections, were rejected. All measurements were made with a Leitz 1/12 homogeneous immersion objective and a Zeiss No. 2 micrometer eyepiece.

A. Influence of light on the position of the red rod nucleus

The object of this series of measurements was to determine the effect of light and darkness upon the position of the nuclei of the red rods with respect to the external limiting membrane.

The nuclei of the rod- and cone-visual cells comprise the external nuclear layer (figs. 1 and 2, *st.nl.ex.*) of the retina. According to Greeff ('00, p. 133), these two kinds of nuclei in amphibians can be distinguished, morphologically, only with great difficulty; hence it is essential to inquire whether any criterion exists whereby the rod nuclei can be identified with a tolerable degree of certainty. The illustrations of the frog's retina given by Greeff ('00, pp. 96, 102) represent the nuclei of the red rod-visual cells as lying considerably nearer the external limiting membrane than the nuclei of either the green rod-visual cells or of the cone-visual cells. If, therefore, in any preparation attention be directed to the nuclei which protrude farthest beyond the external limiting membrane, one may be reasonably sure that the nuclei of the red rods only are under consideration. Ten light-adapted and ten dark-adapted retinas were selected at random for measurement. Not only was considerable regional variability in the position of nuclei found in individual retinas, but also the variability in any limited area was extensive—ranging from nuclei whose edges were at the same level as the external limiting membrane to nuclei which were $3.0\ \mu$ above that membrane. Partly for this reason, and partly to be certain that only the

nuclei of red rods were under observation, attention was directed solely to the mean distance to which the maximally extended nuclei protruded beyond the external limiting membrane.

The following values (grand means) were obtained: in light (10 retinas), 2.9μ ; in darkness (10 retinas), 2.2μ .

On account of the degree of variability observed, I do not regard this slight difference, which, incidentally, is not in agreement with the results of Stort ('87) and Garten ('07) on Triton, or of Angelucci ('90) on the salamander, as indicative of a photic influence. Since the condition recorded in Triton and the salamander is not clearly demonstrable in my preparations of the frog, it follows that whatever changes are found in the rod myoid will be due chiefly to the activity of the myoid itself.

B. Influence of light on the length of the rod myoid. Relative changes at center and periphery of retina

a. Red Rods. In every retina, at least ten measurements were made, on each side of, and not far from, the optic nerve. The averages of these twenty or more values are given in tables 1 and 2 as central measurements. Similarly the means of at least twenty measurements (ten on each side), made well toward the periphery of the retina, are recorded as peripheral measurements. In order to avoid unconscious selection all measurements were made on consecutively-placed rods. The myoid length in the tables constitutes the distance from the external limiting membrane to the nearer edge of the rod ellipsoid (figs. 1 and 2, *my. bac.rb.*).

The tables show that there is considerable variation in individual retinas, yet the rod myoid unmistakably elongates in the light and shortens in darkness (figs. 1 and 2). Although the mean values, at the center and periphery of individual retinas, may also vary greatly in either set, the grand means are practically identical.

The measurements of individual light- and dark-adapted rods overlap in many instances; furthermore, the mean for certain groups of ten deviates considerably from the average for both groups of ten, which constitutes the central or peripheral values

of the tables, as the case may be. In such instances the process of averaging two groups of ten serves to mask this condition, hence the tables, for the sake of compactness, are faulty in this respect.

The conclusion follows, therefore, that the photomechanical responses of the frog's red visual-rod myoid are in agreement with those of other vertebrates in which changes have been demonstrated.

The older workers (Angelucci '84; '90; Gradenigro, '85; and Arcoleo '90) perhaps erred, either in not making actual measure-

TABLE I

Measurements from twenty-three dark-adapted retinas of Rana pipiens. The values are in micra and represent measurements taken along axes coinciding with radii of the eyeball. Each value for the length of the red rod myoid is the mean obtained from twenty consecutively-placed elements

NUMBER OF ANIMAL	NERVE FIBER LAYER TO INTERNAL LIMITING MEMBRANE	CHOROID TO INTERNAL LIMITING MEMBRANE	LENGTH OF RED ROD MYOID AT CENTER OF OPTICA	LENGTH OF RED ROD MYOID AT PERIPHERY OF OPTICA
1	107	64	5.0	4.9
2	136	70	4.0	5.7
3	114	72	4.6	3.3
4	114	64	4.4	4.9
5	122	69	6.6	7.0
6	110	69	5.3	6.7
7	100	64	4.0	6.4
8	106	64	6.7	6.6
9	129	67	6.4	6.4
10	100	69	9.3	7.6
11	114	69	6.9	6.9
12	129	69	5.9	5.7
13	114	73	6.2	5.7
14	97	62	3.6	4.3
15	97	63	4.7	5.0
16	111	69	4.6	5.1
17	107	63	5.4	6.0
18	107	64	6.4	6.2
19	114	63	6.6	6.4
20	103	60	7.2	6.9
21	89	57	7.2	6.3
22	93	62	5.7	7.2
23	93	60	6.4	6.2
Mean.....	109	66	5.8	6.0

ments, or were influenced by their observations on the more strikingly mobile cone (figs. 1 and 2; *my.con.*), the movements of which are the reverse of those exhibited (such is my belief) by mobile rods in general.

In recent papers ('15; '16) the writer favored the anomalous photomechanical responses of the frog's rod, reported by the several older workers, as being more trustworthy than the somewhat confusing account of Lederer³ ('08). The writer ('15),

TABLE 2

Measurements from twenty-three light-adapted retinas of Rana pipiens. The values are in micra and represent measurements taken along axes coinciding with radii of the eyeball. Each value for the length of the rod rod myoid is the mean obtained from twenty consecutively-placed elements

NUMBER OF ANIMAL	NERVE FIBER LAYER TO EXTERNAL LIMITING MEMBRANE	CHORIOID TO EXTERNAL LIMITING MEMBRANE	LENGTH OF RED ROD MYOID AT CENTER OF RETINA	LENGTH OF RED ROD MYOID AT PERIPHERY OF RETINA
1	100	67	9.9	10.2
2	111	73	13.6	14.9
3	93	74	11.9	12.0
4	129	62	11.7	12.6
5	117	72	11.9	12.6
6	114	72	12.6	11.9
7	114	64	13.3	12.2
8	127	64	9.2	12.2
9	127	69	8.6	10.3
10	119	72	16.9	16.2
11	114	72	13.9	14.7
12	120	72	14.3	14.7
13	92	62	9.9	9.2
14	102	69	13.4	13.0
15	107	60	13.0	11.9
16	125	63	9.6	9.2
17	100	62	11.4	12.4
18	106	62	9.9	9.6
19	93	61	8.6	7.9
20	96	69	12.7	9.6
21	107	64	7.9	7.2
22	97	64	8.6	9.7
23	129	69	11.9	12.9
Mean.....	100	67	11.5	11.6

³ Lederer's figures of isolated rods do not necessarily show how long the rod myoid really was, either in darkness or in light. Presumably, the myoid, as the result of teasing, broke approximately at the level of the external limiting

furthermore, made use of these data in an argument against the feasibility of attempting to advance (in the light of our present knowledge) a single rational explanation for the diverse photomechanical movements of the visual rods. It is evident, however, that the conclusion reached in the present investigation renders this particular objection invalid.

It is reasonable to expect, although material is not available at present to put the matter to an experimental test, that the photomechanical behavior of the toad's visual rod will be found to vary in no essential detail from that herein described for the frog.

b. Green rods. From the sixty retinas experimented upon, the ten light-adapted and ten dark-adapted preparations which showed the most perfect histological preservation were selected for measurement. In each retina, measurements were made of the myoid length (figs. 1 and 2, *my.bac.wr.*) of ten consecutively-placed green rods; the results are recorded in tables 3 and 4.

TABLE 3

Measurements from ten dark-adapted retinas of Rana pipiens. The values are in micra and represent measurements taken along axes coinciding with radii of the eyeball. Each value for the length of the green rod myoid is the mean obtained from ten consecutively-placed elements

NUMBER OF ANIMAL	NERVE FIBER LAYER TO INTERNAL LIMITING MEMBRANE	DISTANCE TO EXTERNAL LIMITING MEMBRANE	LENGTH OF GREEN ROD MYOID
1	95	66	21.4
2	94	65	23.0
3	98	75	28.6
4	90	69	26.2
5	112	68	22.5
6	135	82	27.7
7	102	63	24.4
8	109	70	24.0
9	105	70	24.1
10	115	72	22.5
Mean.....	106	70	24.4

membrane, although nothing to this effect is stated. Furthermore, the varicose and atypical appearing rods naturally increases one's caution in accepting his conclusions. Although Lederer gave but little emphasis to his observations on sectioned material (p. 431), I believe that those observations constitute the strongest evidence in support of his thesis.

TABLE 4

Measurements from ten light-adapted retinas of *Rana pipiens*. The values are in micra and represent measurements taken along axes coinciding with radii of the eyeball. Each value for the length of the green rod myoid is the mean obtained from ten consecutively-placed elements

NUMBER OF ANIMAL	NERVE FIBER LAYER TO EXTERNAL LIMITING MEMBRANE	CHOROID TO EXTERNAL LIMITING MEMBRANE	LENGTH OF GREEN ROD MYOID
1	105	68	25.0
2	102	72	26.1
3	127	75	28.2
4	120	69	28.2
5	97	68	31.0
6	112	63	28.2
7	127	87	25.5
8	120	75	27.7
9	127	72	32.4
10	123	66	24.9
Mean.....	116	72	27.7

The tables show that although the difference in the length of the rod myoid, in darkness and in light, is small, the length of the light-adapted element is quite consistently the greater (figs. 1 and 2); hence it seems probable that this difference is significant and the photomechanical responses of the red and green rods are analogous.

This conclusion is not in accord with the results of Angelucci ('94). Angelucci believed that the red and green rod myoids responded similarly to photic stimulation, yet in both cases a shortening was said to take place.

C. Influence of light on the diameter of the red rod

From my preparations many rods were measured, yet no constant or significant differences were found in the diameters of the outer members (figs. 1 and 2, *prs.dst.bac.rb.*), or of the ellipsoids (figs. 1 and 2, *ell.bac.rb.*) of the inner members, which could be attributed to the influence of light and darkness. The myoid of the inner member (figs. 1 and 2, *my.bac.rb.*) naturally becomes tenuous when elongated by the action of light (p. 436).

One is inclined to question the morphological normality of the teased rods figured by Lederer ('08), especially since the appear-

ance of these elements in his own sectioned material was dissimilar. In the figures corresponding to the text descriptions of dark-adapted rods (the figures and their appended descriptions are apparently interchanged in his paper), the varicose outer members are twice, and the ellipsoids three to four times, as broad as the corresponding parts of the light-adapted rods.

My observations, therefore, are opposed both to those of Lederer ('08) who believed that darkness causes the rod to swell, and to those of Ewald and Kühne ('78), who recorded that light acts in this manner.

SUMMARY

1. Distinct movements of the nuclei of the red rod-visual cells, due to photic stimulation, are not demonstrable. Hence movements of the rods are not produced indirectly in this way.

2. The myoid of the rod-visual cell elongates in light and shortens in darkness. *Therefore, contrary to the conclusions of the older workers, the photomechanical response of the frog's rod myoid is found to be similar to that occurring in the retinas of all other investigated vertebrates.*

The mean length of approximately 1000 myoids from 23 light-adapted retinas is $11.6\ \mu$.

The mean length of approximately 1000 myoids from 23 dark-adapted retinas is $5.9\ \mu$.

3. A significant regional difference in the length of the red-visual rod myoid is not apparent from comparisons of approximately 2000 measurements made at the center and the periphery of light- and dark-adapted retinas.

4. The myoid of the green visual rod probably elongates slightly in the light and shortens in darkness.

The mean length of 100 myoids from 10 light-adapted retinas is $27.7\ \mu$.

The mean length of 100 myoids from 10 dark-adapted retinas is $24.4\ \mu$.

5. Definite changes in the diameter of the outer member, or of the ellipsoid, of the red visual-rod can not be correlated with photic influences. The rod myoid, however, does become tenuous in the light.

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A PRELIMINARY DETERMINATION OF THE PART PLAYED BY MYELIN IN REDUCING THE WATER CONTENT OF THE MAMMALIAN NERVOUS SYSTEM (ALBINO RAT)

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ONE CHART

It is a familiar fact that there is a progressive loss of water in the brain and spinal cord with advancing age. This is illustrated for the albino rat in Chart 1.

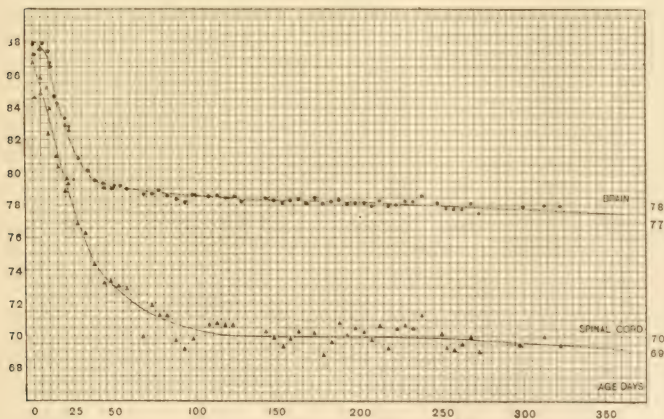


Chart 1 Showing the percentage of water on age in the central nervous system of the albino rat. The upper graph gives the values for the water in the brain as determined by the formulas (Hatai, in 'The Rat,' Donaldson, '15). The lower graph gives the corresponding values for the spinal cord, determined in the same way.

The small black dots indicate the observed values for the several age groups for the brain, and form the data for the formulas. The small black triangles have a like value in relation to the spinal cord.

It is also well known that in some mammals all the axons in the central nervous system are unmyelinated at birth, while in other species a greater or smaller number of them may already have their sheaths. In all cases, however, an active formation of myelin occurs during the period of rapid growth (Koch and Koch '13).

In a previous study on the percentage of water in the albino rat I was misled by certain graphs (Donaldson '10) to the conclusion that probably both the axons and their myelin sheaths changed in their water content so as to produce the well known reduction of water which is observed, but further study has shown that this is an incorrect view, and it is the object of this paper to present the evidence of my revised conclusion.

Since it has already been shown that the loss of water in the human brain follows the same course as in the brain of the albino rat—and has similar limits (Donaldson '10)—it is permissible to use in the argument certain observations on the human brain.

From the data for the human brain already in the literature I have selected those published by de Regibus ('84), because this author evidently examined only the outer layers of the cortex when making his determinations for the water in the gray substance and because he was able also to obtain remarkably uniform results for all of his determinations.

De Regibus tested four male brains, 25 to 76 years of age and three female brains, 30 to 60 years of age.

In Table 1 there appear also determinations of the water content in the human cortex and in the fibers at birth. These are based on the records of Weisbach ('68).

This enables us to contrast in Table 1 the conditions at birth with those at maturity.

TABLE 1

Percentage of water in the gray and white substance of the human brain at birth and at maturity

	CORTEX (GRAY)	CALLOSUM (WHITE)
	<i>per cent</i>	<i>per cent</i>
At birth (Weisbach).....	88	88
At maturity (de Regibus).....	86	70.4

According to this table the (gray) cortex has lost 2 points and the (white) callosum 17.6 points in the process of maturing.

It is never possible at maturity to obtain the cortex or any other gray mass without some admixture of myelinated fibers and I have therefore, provisionally, credited one point of the loss, noted by de Regibus in the water content of the cortex, to the presence of myelin. This implies but a small proportion of myelin since if we assume that myelin has 48 per cent of water the reduction of 1 per cent would mean that about one thirty-ninth of the mass was represented by myelin. According to this assumption the mature gray substance (cortex)—when the myelin is excluded—contains 87 per cent of water, and in the computations which follow the neurons without myelin are assumed to have 87 per cent of water, except at ten days, when they are credited with 88 per cent.

The fact that the fibers without myelin have at birth a high percentage of water (88%) while at maturity, after myelination, they have lost 17.6 points is the sort of evidence which furnishes the basis for the current, but unsupported view, that the loss of water is to be associated with the formation of the myelin. It is our object to present more precise information bearing on this point.

To obtain a notion of the approximate distribution of the water between the myelin and neurons proper, it is necessary to have data on the relative abundance of these two constituents of the brain.

In 1913 W. Koch and M. L. Koch made a study of the chemical composition of the brain of the albino rat at six ages between birth and maturity, and of the spinal cord at one age. The data thus obtained are those which will be utilized here. The authors determined seven fractions: Proteins, organic extractives and inorganic constituents, which three taken together, we shall designate protein (or non-lipoid), and phosphatides, cerebro-sides, sulphatides and cholesterol, which four taken together, we shall designate lipid.

These data give us at each age, therefore, the protein and the lipid present in the brain, or to be a little more exact, we should

say the lipid and the non-lipoid fractions. The lipid (in part) represents the myelin sheaths, while the protein, with the remainder of the lipid, represents the cell bodies and their unsheathed axons.

With the exception of the one day group, the ages for which analyses were made, are given in table 2.

TABLE 2

To show, for the brain of the albino rat at five ages and for the spinal cord at one age the percentage of water in the myelin as computed according to the method described. The protein and lipid are given in percentages of the total dry substance. (Based on table 2. Koch and Koch '13)

Brain

(1) AGE IN DAYS	(2) CORRECTED PROTEIN	(3) CORRECTED LIPOID	(4) PROPORTION OF LIPOID FOR DIFFERENT AGES. LIPOID AT 20 DAYS = 1	(5) (6) PERCENTAGE OF WATER		(7) WATER, IN MYELIN = LIPOIDS (C) (COMPUTED) (See p. 447)
				Entire brain observed	In Neurons = protein (C) (assumed)	
10	<i>per cent</i> 93.80	<i>per cent</i> 6.2		86.5	88	63.8 ¹
20	88.88	11.12	1.0	82.5	87	46.5 ²
40	82.86	17.14	1.5	79.4	87	42.7
120	75.11	24.84	2.2	78.4	87	52.4
210	76.36	23.63	2.1	78.1	87	47.4
Average of 20 to 210 days.....						47.8

Spinal cord

120	52.92	47.08	4.2	70.4	87	51.0
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¹ First traces of myelin.

² Myelin well shown.

At one day—or practically birth—it is found that the lipid is present to the extent of 0.31 or nearly one-third of the weight of the protein. There is, however, no visible myelin at this age, so it is concluded that this proportion of the total lipid is normally associated with the protein and is not to be included in the lipid which forms the myelin sheaths. We have treated the data for the later age groups in accordance with this relation, and in each case have taken from the total lipid found an

amount equal to 0.31 of the protein found. The remaining amount of lipid is assumed to be that used for the sheaths.

In table 2, the column (2) headed Corrected Protein gives the observed protein (non-lipoid) plus 0.31 of itself and the column (3) headed Corrected Lipoid gives the observed lipid less the amount of lipid added to the protein.

In table 2 the data are given in five age groups for the brain and in one age group for the spinal cord. It is to be noted that the 10 day brain group—which stands just at the beginning of the myelin formation—is for the moment excluded from the discussion and we begin the comparisons which are to be made, with the 20 day brain group.

In the brain series (with one exception) the corrected protein diminishes and the corrected lipid increases with advancing age. Between 20 and 210 days the proportion of the lipid doubles—column (4). We have in column (5) the observed percentage of water in the brain as a whole. It is assumed, as previously noted, that the corrected protein (neurons in the strict sense = both cell bodies and axons) have 87 per cent of water. From these several data we can compute the percentage of water to be assigned to the corrected lipid, which represents the myelin.¹

The method of computation may be illustrated by the data for the 20 day group. Reference to table 2 shows that at this age there is 1 part of lipid (11.12%) to 8 parts of protein (88.88%). This gives 9 parts, representing the entire brain and having 82.5 per cent of water. The product, $9 \times 82.5 = 742.5$. We assume that the 8 parts of protein have 87 per cent of water. The product, $8 \times 87 = 696$. The 1 part of lipid, representing the myelin, will then have a percentage of water equal to the difference of these products = $742.5 - 696 = 46.5$ per cent.

¹It is hardly necessary to point out that a division of the brain into myelin on the one hand and neurons on the other fails to enumerate several structural elements which are also present though representing a relatively small mass. There are, in addition to the neurons, glia and ependymal cells; blood and lymph vessels; blood and lymph and a slight amount of connective tissue. Over against the neurons plus this group of elements we put the myelin, but for the purposes of the present discussion it is convenient to speak of the rest—the non-myelin portion—of the brain as if represented by the neurons alone.

As table 2 shows, similar computations give percentages of water for the myelin in the brain, which range from 42.7 per cent to 52.4 per cent and which yield a mean value of 47.8 per cent. The computation for the spinal cord gives 51 per cent. The significance of these results lies not in the particular percentage of water here determined for the myelin—as that depends somewhat on the percentage of water assumed for the protein—but on the similarity of the values found in all the five cases examined.

However, it is found on trial that one cannot depart far from the value of 87 per cent for the proteins without obtaining rather improbable percentages for the myelin, so that this value is probably nearly right.

DISCUSSION

Those familiar with the published data for the percentage of water in the cortex will at once perceive that the value given by de Regibus (86%) seems high. The various water records for the cortex run down as low as 83.5 per cent. The differences between the various determinations are, however, almost certainly due to the varying amounts of white substance included in the sample, and as has already been stated the value chosen is probably close to the true value.

In connection with the computations there are, however, two conditions which have been assumed to be constant but which in all probability, are subject to variation. I refer to the density of the myelin and to the fraction of the lipoid to be assigned to the protein. As to this last condition, it would be plausible to think of a larger fraction of lipoid in the axons than in the cell bodies. If this were true it would be necessary to increase this fraction in the case of the spinal cord or the callosum.

It is also possible that aside from this method of distribution the fraction of lipoid in the neuron may increase with age. The slight loss of water during the first ten days of (rat) life is possibly due to such an increase. Finally, the density of the myelin may change with age, as its chemical composition certainly does, and it is conceivable that it has a higher water content when first formed, as is suggested by the 63.8 per cent given for the ten day record in table 2.

If we compare the drawings of Watson ('03), showing the increase of the visible myelin in the cerebral hemispheres and in the spinal cord of the rat, with the chemical results here used, we see that the histological pictures show a more gradual appearance of myelin than the chemical results, or the water determinations, would suggest. This probably depends on the fact that it is only a fraction of the lipoids forming the sheaths, which takes the haematoxylin stain, and this stainable fraction forms at first a smaller, but later a larger portion of the entire sheath (Koch and Koch '13; Smith and Mair '08).

There is still one more modification in the formation of myelin. Tribot ('05) has contrasted in terms of dry substance the relative amounts of albuminoides and the fats in the nerve tissue of the guinea-pig at different ages. The percentage value of the fats increases from 11 days (his first observation) up to 120 days—after which it begins to fall. The fats of Tribot are the lipoids of Koch's analysis and it is of interest to note that the 120 day record for the brain in table 2, column (4), also shows the highest proportion of corrected lipoids. The observations of Dunn ('12) which show in the myelinated fibers of the second cervical nerve of the rat the highest relative areas for the myelin sheaths at 75 days and 132 days—seem to fit with these other observations and to suggest that the formation of lipoids with advancing age fluctuates in such a way as to show a maximum about the end of the active growing period of the central nervous system.

This discussion of possible factors modifying these determinations has been introduced to clear the way for further work on the main question, but so far as one can foresee the effect of taking them into consideration, it would tend to make more uniform the values thus far obtained.

CONCLUSIONS

We conclude from these results that there is no evidence that the cell bodies and their unsheathed axons suffer more than a slight loss of water between birth and maturity, and that the progressive diminution in the water content of the entire brain and spinal cord is mainly due to the accumulation of myelin,

with a water content of about 48 per cent. Moreover, the myelin must be regarded as a more or less extraneous substance, having but little significance for the characteristic activities of the neurons.

If we compare the loss of water in the case of the nervous system with that in the muscular system, which also contains a large proportion of fat (Tribot '05), we find that while the two systems lose about the same percentage of water between birth and maturity (Lowrey '13), yet in the case of the nervous system alone is this lipoid (or non-protein substance) accumulated outside of the cell. From this it is seen that the neuron is peculiarly able to maintain its early water-solids composition and that it accomplishes this by throwing out the material, which in the muscles is retained within the cells.

As the diminution in the percentage of water in the central nervous system is preëminently a function of age, and as it appears to be due almost entirely to the formation of the myelin, it follows that the myelin formation is also a function of age (Donaldson '11). A glance at the graphs in Chart 1 shows that the most active production of myelin, as indicated by the rapid loss in the percentage of water, occurs early, i.e., during the first forty days of rat life, in the brain, and during the first hundred days, in the spinal cord.

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THE TASTE OF ACIDS¹

W. J. CROZIER

TWO FIGURES

I. The problem of irritability is essentially a matter concerning sense organs; in these structures we find cells "as nearly as possible unifunctional" (Lucas, '09, p. 328) with respect to the capacity for being stimulated. Hence the analysis of irritability should properly be founded upon the study of receptor physiology in higher animals, rather than upon an examination of the properties of protoplasm in deceptively simple protozoans.

Two contrasted methods have in the past been used in the study of stimulation. The first of these deals with the responses of unicellular organisms, together with the (usually vague) application of principles derived from cells of more or less generalized type. The second method has consisted in the analysis of sensations. Yet, if we consider such questions as those involved in taste stimulation, we find that no definite conclusions have been reached. The very complexity of organization in 'simple' forms is probably in the main responsible for the results obtained in the first field, results which we find stated in such weak generalizations as occur in Verworn's book ('13, pp. 41, 86, 94). The second procedure, the study of human sense organs, has inevitably come to be clouded by psychological interpretations.

It appears that the possibility of arriving at some understanding of what occurs in stimulation has not yet been sufficiently tested out from the standpoint of sense organ irritability as revealed in animal reactions. This paper is a contribution toward that end; it deals with the problem presented by the taste of acids. I am indebted to Prof. G. H. Parker for his criticism of the manuscript.

¹ Contributions from the Bermuda Biological Station for Research, No. 46.

II. Practically the whole of the difficulty is contained in the often considered case of acetic and hydrochloric acids. A comparative study of the reactions to these and other acids as given by the earthworm (Hurwitz, '10; Crozier, '16), leads to a preliminary simplification of the matter, namely to the formulation of two separate problems: these acids stimulate, they also result in a sour taste. These two problems must be considered independently; we must distinguish between (a) that property of an acid which causes it to be efficient in stimulation and (b) that which determines the sourness of an acid solution.

The relative efficiency of an acid as an agent of stimulation may be measured by the liminal effective dilution. The point I wish to make is that the property of hydrochloric acid which causes it to be tasted in solutions about five times as dilute as that limiting the tastability of acetic acid may have no immediate connection with the common cause of the sourness perceived when these acids act upon the tongue.

This point of view contains the possibility of an explanation for (and is, reciprocally, in part justified by) the fact that acids exhibit a characteristic astringency in solutions so dilute that they are no longer sour. There is some evidence, also, that the sourness and astringency may be separated experimentally, as by the use of cocaine. There is therefore ground for the opinion that in taste excitation by acids two processes occur, and that the production of a sour taste is the secondary one.

The points which require solution with respect to human taste excitation by acetic and hydrochloric acids are:

- (a) Both these substances stimulate.
- (b) They stimulate in different degrees, in dilute solutions the stimulus from hydrochloric acid being the more intense.
- (c) They both result in a sour taste.
- (d) They fail to stimulate when too greatly diluted.
- (e) The dilutions which limit the capacity of the two acids to stimulate are very different ($\text{HCl } n/900 \approx$; acetic, $n/200 \approx$).

Each of these points—except (c)—is closely paralleled by the details of earthworm reactions to acids (Crozier, '16), and also

by the reactions of *Æolosoma*, a freshwater oligochaet (Kribs, '10).

It adds somewhat to the clearness of the discussion if, as I have previously proposed (Crozier, '14, p. 16), we restrict the word 'stimulus' to mean the change induced in a receptor by the action of a stimulating agent. The explanation of the acid taste is made easier by the fact that one is not called upon to account for a sour taste resulting from heterologous stimulation; there is no good evidence for the existence of a sour taste not directly produced by acid. The extreme specialization of the acid taste makes it a very favorable case for analysis.

III. It is sufficiently obvious that only the surface of the receptor is immediately concerned in stimulation. This view is reasonable upon purely morphological grounds, such as the modifications of the exposed ends of sensory cells² and the relations of nerve fibrils to the surface of secondary sense cells. The way in which this conception of the cell surface as the organ of irritability has been elaborated by R. S. Lillie and others need not be discussed here. But it may be pointed out that the method of interpreting mechanical excitation has been indicated by Osterhout ('15) and by such observations as that of Evans and Winternitz (Evans and Schulemann, '14, p. 453). Unequivocal evidence in this direction is afforded by Harvey's work on the penetration of cells by alkalies, (Harvey, '14 c), by his experiments with acids (Harvey, '14 c), and by my own study of this latter subject (Crozier, '15, '16); the evidence referred to is, in brief, to the effect that penetration of the body of the cell substance is altogether too slow a process to account for the rapidity of taste stimulation. The slowness of acid penetration is due to the resistance offered by the cell surface, a resistance which practically disappears with the death of the cell. An examination of cell penetration by various acids, employing a wide range of dilutions, should therefore yield valuable information regarding the composition and behavior of the resistant cell surface. In several cases a study of this

² The probable chemoreceptors of the earthworm have been described by Miss Langdon ('95) and Bovard ('04).

kind has been made possible by the discovery of intracellular indicators sensitive toward acids (Harvey, '14 b, Crozier, '15).

Measurements of cell penetration by acids show, so far as concerns the present problem: 1) that both acetic and hydrochloric acids are able to penetrate the cell; 2) other things being

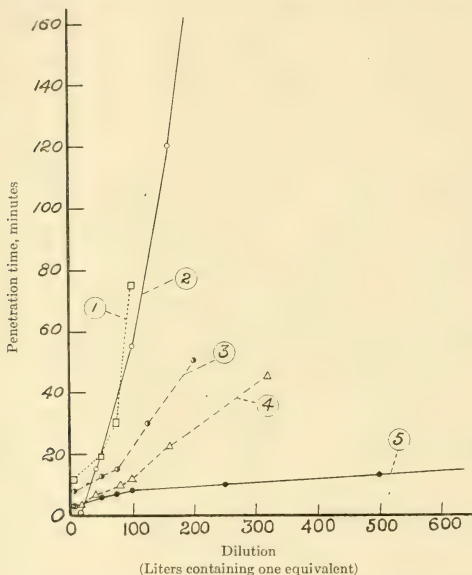


Fig. 1 The average time of penetration of cells by hydrochloric, butyric, and acetic acids.

- | | |
|----------------------|---|
| 1, Acetic acid | } integument of <i>Chromodoris zebra</i> , a nudibranch. |
| 3, Butyric acid | |
| 5, Hydrochloric acid | |
| 2, Butyric acid | } testis epithelium of <i>Stichopus ananus</i> , a holothurian. |
| 4, Hydrochloric acid | |
- 1, 3, 5, from Crozier ('16), at 27°.
2, 4, from Harvey ('14 c), at 28°.

Butyric acid is introduced for comparison, since Harvey (loc. cit.) gives no data for dilutions of acetic; the principle is the same for both these acids. It should be noted that in Harvey's experiments the acid was dissolved in a balanced salt solution, in mine in distilled water.

equal, the speed of this penetration varies with the acid and its concentration; 3) the curves relating penetration time to concentration are different for the two acids, dilute solutions of hydrochloric penetrating much more easily than acetic. Points of resemblance, in fact of identity, are found among the characteristics of stimulation by these acids, and have been enumerated as a, b, e, in section II. The closeness of the parallelism indicates that the stimulus is due to the penetration of the surface layer of the cell. In stimulation by these acids the essential step is the union of the stimulating agent with some constituent of the ceptor surface.

According to Becker und Herzog ('07), the decreasing order of intensity of taste sensation due to the acids they tested was HCl , HNO_3 , trichloroacetic, formic, lactic, acetic, butyric. These acids penetrate the indicator-containing cells of *Chromodoris zebra* in the order HCl , HNO_3 , formic, monochloroacetic, lactic, butyric, acetic (considering a range of concentrations; Crozier, '16). There is reason to believe that trichloroacetic penetrates more speedily than monochloroacetic; and with this in mind, the parallelism between these two series becomes surprisingly close. This particular case is quoted with the purpose of pointing out that a small series of acids, such as that just cited, may to all appearances support the view that the intensity of taste excitation is proportional to the ionization strength of the acids; similarly, a penetration series may be chosen which seems to prove a corresponding relation. Yet, further examination of a large number of acids shows that ionization is but one of the factors determining cell penetrating power, and the existence of influences in addition to ionization has also been suspected with reference to acid stimulation; direct evidence upon this point has hitherto been lacking.

IV. Analysis of penetration data obtained with eighteen acids has shown (Crozier, '16) that in any given case ability to penetrate the cell appears to depend upon two (or more) factors: one is the ionization strength of the acid; the second depends upon some other aspect of the acid molecule. To account for these results it is further necessary to assume that the cell surface is

of heterogeneous constitution. This conclusion may be extended to include the surface of chemoreceptors, since it enables us to account not only for the reactions of the earthworm to dilutions of acids (Crozier, '16), but also clears up a well known anomaly in regard to human taste.

It was shown by Richards ('98) and by Kahlenberg ('98) that the hydrogen ion must in some way be responsible for the sour taste. Yet acetic acid, which ceases to be tasted at dilutions

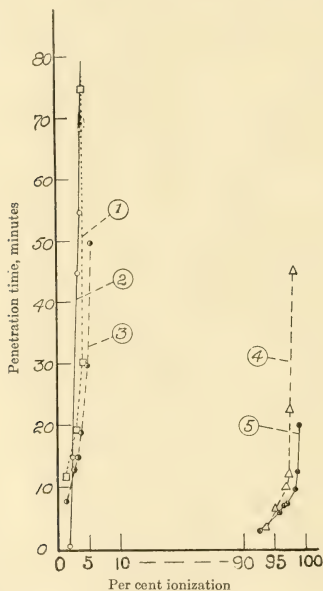


Fig. 2 The relation between percentage ionization and time of cell penetration from solutions of hydrochloric, butyric, and acetic acids.

- | | |
|----------------------|--|
| 1, Acetic acid | } integument of <i>Chromodoris zebra</i> . |
| 3, Butyric acid | |
| 5, Hydrochloric acid | |
| 2, Butyric acid | } testis epithelium of <i>Stichopus ananus</i> . |
| 4, Hydrochloric acid | |

1, 3, 5, from Crozier ('16).

2, 4, from Harvey ('14c).

below about $n/200$ is only 6 per cent dissociated at that concentration, whereas hydrochloric acid, fully dissociated (99 per cent), is tasted down to about $n/900$. The actual hydrogen ion concentrations in these solutions limiting tastability are: HCl ($n/900$), $C_H = 0.00119$ N; acetic ($n/200$), $C_H = 0.00035$ N. Acetic acid is more stimulating than would be calculated from its dissociation.

Exactly similar conditions are found in studying the penetration of cells by acids, as will be clear from an inspection of the two sets of curves in figure 2. In table 1 acid solutions giving

TABLE 1

The ionization of hydrochloric, acetic, and butyric acids, in solutions which penetrate cells within equal times. For sources of data see corresponding reference numbers in figure 2.

TIME OF PENETRATION, MINUTES	IONIZATION, PER CENT				
	Hydrochloric		Acetic	Butyric	
	[5]	[4]	[1]	[3]	[2]
5	95.0	94.5			2.0
10	98.5	96.7		2.0	2.1
15	98.8	97.4	2.1	3.3	2.5
20	99.0	97.5	3.0	4.0	2.6
50			4.0	5.5	3.5

the same penetration time appear in the same horizontal row; it is seen that the penetration of cells by acetic acid is much more efficient than if its dissociation were the deciding influence.

It follows that the stimulus produced by acetic acid is due, in the first place, to its union with the cell surface; this penetration of the plasma membrane is more efficient than if dissociation strength were the determining factor. The indication is that this acid dissolves in a fatty substance located at the cell surface (for further evidence, see Crozier, '16). The stimulus due to hydrochloric acid also depends upon its penetration of the cell surface, but there is some evidence that it involves proteins.

It is not clear to what extent stimulation by acetic and hydrochloric acids may be interpreted in terms of the effects which

these substances have upon surface colloidal conditions, and thus upon cell permeability (Spaeth, '16). Herlitzka ('10) has discussed this question with reference to the taste of salts, but measurements of the effect of acid on permeability (Osterhout, '14) have not considered the possibility that characteristically different results may follow from the action of diverse acids; Osterhout's ('14) measurements deal only with hydrochloric acid.

V. There remains to be accounted for the production of a unitary taste quality, the sourness of acid solutions. This must be related to ionizable hydrogen (Kahlenberg, '98, Richards, '98). But it has just been shown that hydrogen ions outside the cell surface cannot be the effective agents, since stimulation by these acids parallels so closely the peculiarities of their penetration of the cell. The hydrogen ion must therefore act after the acid has united with the ceptor surface. At least two possibilities are obvious: (1) the presence of potentially ionizable hydrogen within sufficiently concentrated undissociated molecules is enough to produce the sour taste, or (2) the acids ionize secondarily after entering. The first suggestion is not so far fetched as it may at first seem, though it is not in accord with current teaching. Numerous parallels could be drawn from the taste of organic substances (Francis and Fortescue-Brickdale, '08, pp. 331 et seq.). I believe that this view is possibly correct, although something could also be said for the hypothesis of secondary ionization following some reaction involved in gaining admission to the outer layer of the cell. It is in this latter direction that the explanation (Beutner, '14) must be sought for the source of electromotive effects accompanying stimulation in general, which presumably occur also in taste excitation.³ This interpretation disposes of the difficulty (very conspicuous in the case of taste) which is encountered by those who would make the process of stimulation and the production of electromotive effects completely identical in every case.

³ A taste cell bathed by a stimulating solution is in a condition very similar indeed to that of the various tissues experimented upon by Loeb and Beutner (Loeb, '15).

SUMMARY

An attempt has been made to account for the action of acetic and hydrochloric acids upon the sense of taste. It is shown that the stimulus due to these substances probably depends upon their union with diverse constituents of the ceptor surface. It is thus possible to explain, by comparison with the penetration of cells by these acids: how they stimulate at all, why they stimulate to different degrees, and why acetic acid gives a more powerful stimulus than if it acted by dissociation alone. Why these acids produce a unitary (sour) taste quality is a problem of a different order; it is possible that this depends upon the presence of potentially ionizable hydrogen within undissociated acid molecules, though secondary ionization may also play a part.

AGAR'S ISLAND

BERMUDA.

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THE NERVOUS SYSTEM OF PYCNOGONIDS

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TWENTY-ONE FIGURES

The nervous system of pycnogonids presents many peculiarities. It is rather difficult to find the counterpart of this system in other arthropods. The nervous system of some Crustacea suggests it, especially in those forms with an elongated thoracic region and reduced abdomen. The general arrangement of the ganglia is totally unlike the central nervous system of arachnids although the general form of the body of 'sea spiders' strongly suggests arachnid relationships. The rather small supraesophageal ganglion and the well developed chain of ventral ganglia suggest a rather primitive type of nervous system, but the innervation of the pharynx and proboscis presents complex and apparently unique conditions.

Although there is an extensive literature on the classification, structure and development of pycnogonids, there is little or nothing on the structure of the nervous system.

The general form of the ganglia with their chief branches is quite well known, for nearly every paper on the classification of the group contains a more or less detailed sketch of the animals described with the nervous system shown in place.

The supraesophageal ganglion seems to contain but two pairs of ganglia recognized by early authors in other arthropods as the protocerebrum and deutocerebrum, the tritocerebrum found in some arthropods being absent. This is but one of several structures that point to a closer relationship with arachnids than with Crustacea. However, without going into further reasons at this time, I am inclined to side with Dohrn and consider Pycnogonida a separate class.

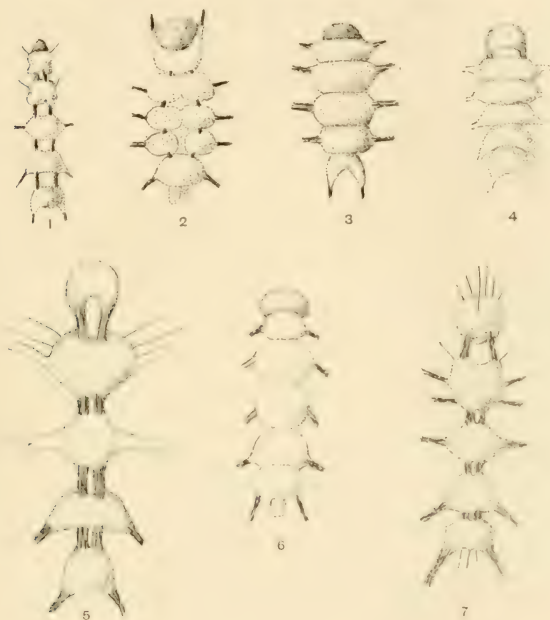
As the tendency has been to regard these animals as arachnids, it may be worth while to glance through the neurological literature on this group.

Among the earliest work on the nervous system of arachnids was that of Treviranus in 1812. No hint of pycnogonids is given in this paper, nor is there any mention of these animals in the work of B. Haller just a century later. There is no reference to pycnogonids in the extensive work of Saint Remy, '90. Dahl, in 1913, gives a brief summary of the work of Dohrn in connection with various types of arachnids. If we go through the extensive literature on the pycnogonids as a group we find, it is true, little of the structure of the nervous system, but much about the arrangement of the ganglia composing it.

From the works of Hoek, '78, '81, Dohrn '70, '81, Sars '91, Meinert '98, and a number of others, as well as from the study of Pacific coast forms, we learn that the central nervous system consists of a supraesophageal ganglion and a ventral chain of from four to five chief ganglia. The smaller number of ganglia we find when the body is less elongate. The supraesophageal ganglion has a ventral median nerve to the proboscis, nerves to the eyes and a pair to the chelifori. Each ventral ganglion has at least one main branch. Three branches from the first ventral ganglion are as follows: 1) A small pair or two pairs to the proboscis; 2) a pair to the palps; 3) a pair to the ovigers; 4) if the first ganglion is fused with the second as it is in those with four ganglia, then there is also a pair to the first pair of walking legs.

Figures 1 to 7 show different types of nervous systems from Pacific species of pycnogonids. The method by which the nervous system was studied by some observers was simply to determine the position of the ganglia through the transparent body-wall. This was tried with a number of specimens after the animals had been fixed in mercuric fluids. In some cases the whole animal was stained and mounted in such a way as to show the internal ganglia. In some cases the animals to be studied were placed for a short time in caustic or acid and by one or the other of these methods the internal parts were

cleared so that the ganglia might be seen. Serial sections of the whole animals were also made for study, but the chitin often makes perfect series impossible. Hoek and possibly



Figs. 1 to 7 Drawings from the adult nervous systems of a number of species of California pycnogonids. The supraesophageal ganglion is shown at the upper end of the figures in every case. The nerves are not all shown in every figure. All are shown from the ventral side, the ganglia were exposed by various methods and all are not drawn to quite the same scale.

Fig. 1 *Eurycyde spinosa*, Hilton.

Fig. 2 *Halosoma viridintestinalis*, Cole.

Fig. 3 *Tantystylum intermedium*, Cole.

Fig. 4 *Ammothella tubercuata*, Cole.

Fig. 5 *Pycnogonium stearnsi*, Ives.

Fig. 6 *Palene californiensis*, Hall.

Fig. 7 *Anophlodaetylus erectus*, Cole.

some others have used gross dissection with the larger species. I also tried this method and found that it was not difficult to expose and remove the whole nervous system from even the smallest specimens. For the structure of the ganglia serial sections were made from these removed ganglia.

There seems to be some difference of opinion as to number and position of abdominal ganglia. There are without doubt ganglia in the adult that may be called abdominal, but they are often not evident or indicated by slight knobs on the last ganglion. Probably in no case are these little ganglia in the abdomen (figs. 1, 2, 6, 7, 19).

The special nerve supply to the proboscis has been described by Hoek, Dohrn and others. I was able to dissect it out in a number of species where I have found essentially the same features already described. In the genus *Pycnogonum* I found a similar condition as shown by Hoek. Practically the same condition was found in two other genera not before described. There are three main branches which run to the three divisions of the proboscis: a dorsal branch running from the mid-ventral line of the supraesophageal ganglion, and two lateral branches springing from the forward part of the first ventral ganglion. Each of these branches has numerous small ganglia along its course and near the end of each branch there is a much larger ganglion. Branches connect the three trunks with each other and fine nerves run from each ganglion to adjacent parts of the proboscis. Lateral to these three ganglionated branches is a more external nerve which sometimes has a separate origin from the larger ganglia or from the ganglionated trunks. These three more superficial branches appear to fuse in places with the deeper branches, but they do not bear ganglia.

This whole complicated structure seems quite unusual and some have seen in this proboscis region the representations of other segments of the animals. However I prefer the assumption of Dohrn that the proboscis represents only a secondary growth of the lips of the stomodoeum. I believe the special nerves of the proboscis represent the system of frontal nerves and ganglia which we find in *Insecta* and other arthropods.

The small ganglia of the proboscis are rather new structures, but the large, represent the frontal and lateral head ganglia of other forms.

The development of the nervous system of pycnogonids has been especially studied by Morgan, '91. and Meisenheimer, '03, although a number of others have studied the general life histories, or special stages. According to Meisenheimer, in the embryos of *Ammonothea* the early development of the ganglia is much as in other arthropods, a longitudinal strip or germ band envelops the yolk and paired thickenings of the ectoderm occur which represent cerebral and post-oral ganglia. I have not followed these earliest stages in any of my material. At the time that the free larva is liberated, there is seen a supraesophageal ganglion and three pairs of sub-intestinal centers such as shown in figure 13 of a California form of the same genus. The second of these two ganglia is composed of two parts and represents the second and third parts fused. This type of larval nervous system seems rather typical of this sort of larval form. What the changes are from this to the adult are not exactly known, but suggestions may be obtained from the study of other species. Morgan in *Tanystylum* gives some idea of the gradual development of additional ganglia in the caudal region as the larva with three pairs of appendages add a fourth and a fifth pair successively and later a sixth pair. At this last period the maximum number of ganglia is attained, this number becomes reduced with the growth of the seventh pair of appendages and the adult condition is reached. During the early stages the addition of extra ganglia is probably not so much from the backward growth of nervous tissue as from later developments from the surface. In *Palene* the type of development is different because of the large yolk mass. Separate ganglia for the segments are developed, each of these has at an early period an invagination or 'ventral organ.'

The species whose development I have especially studied seems intermediate between the free living larval form and such a continuous type of development as shown by *Palene*. This genus *Anoplodactylus* is more parasitic during larval stages

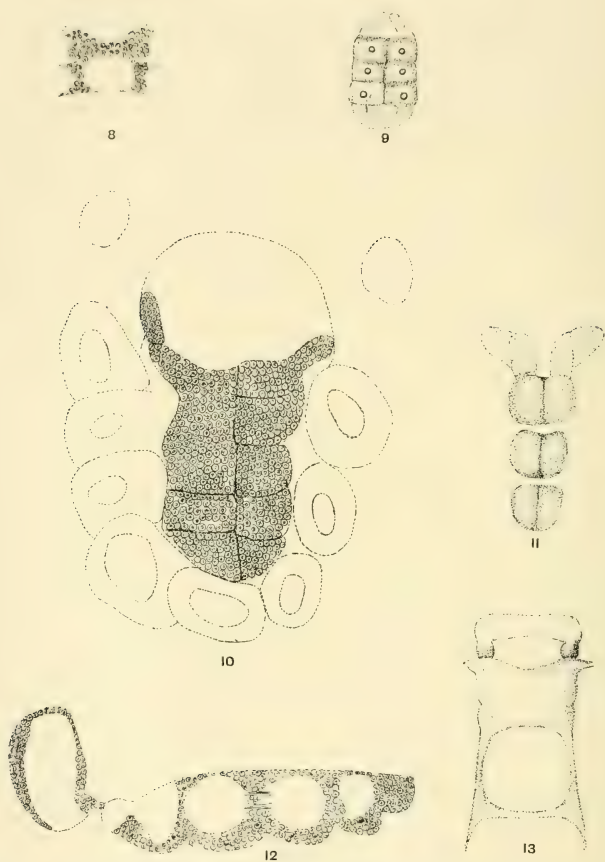


Fig. 8 Central nervous system of *A. erectus* during first larval stage. $\times 350$.
 Fig. 9 Nervous system of *A. erectus* during the second larval period. $\times 350$.
 Fig. 10 Section through whole larva of *A. erectus* during the third larval period. $\times 350$.

Fig. 11 Central nervous system of *A. erectus* at about the third larval stage. Drawn from a whole mount which did not show as much as some others. $\times 300$.

Fig. 12 Longitudinal section through the central nervous system of *A. erectus* during the last larval stage. $\times 350$.

Fig. 13 The central nervous system of the first larval stage of *Ammothella*. $\times 350$.

than the others mentioned. The first larval stage soon attaches itself to, and enters hydroids. It has three appendages in the first larval stage, one pair is chelate, the last two have long tendrill-like extensions. At such a period the nervous system is not easily made out from surface views, but it is much like that of *Ammothella*. Figure 8 shows three parts, a larger thicker portion which has nerves to the larger first appendages, and on each side back of this a group of cells corresponding to the other appendages. A moult within the hydroid gives rise to a small larval form without the long appendages and it is at

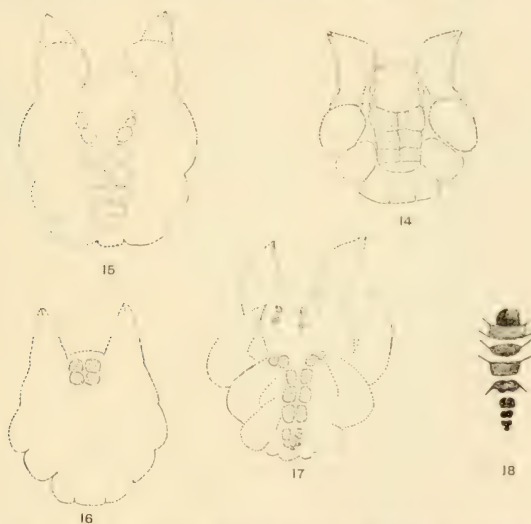


Fig. 14 Outline of ventral view of larva and ganglia from below *A. erectus*, third larval stage. $\times 85$.

Fig. 15 Outline of a ventral view of a later stage larva than figure 14 of *A. erectus*. $\times 85$.

Fig. 16 Outline of a dorsal view of a larva of *A. erectus* about the same stage as figure 14. The brain is shown. $\times 85$.

Fig. 17 Fourth stage larva of *A. erectus* from below. $\times 35$.

Fig. 18 Central ganglia of a larva of *A. erectus* with three pairs of walking legs. The drawing is from below. The upper area without nerves in the figure is the supraesophageal ganglion. $\times 35$.

such a period that new ganglionic material seems to be developed. Figures 9, 11 and 14 are drawn from such early stages. At a later moult more ganglia are evident, as in figures 10, 15, and 16. The ventral ganglia at first are mere groups of cells, as is shown in the frontal section from which figure 10 was taken. As may be seen from the figures 10 and 15, ganglia are developed in each segment, a pair for each appendage and several for the cephalic region and a common mass of cells for the ab-

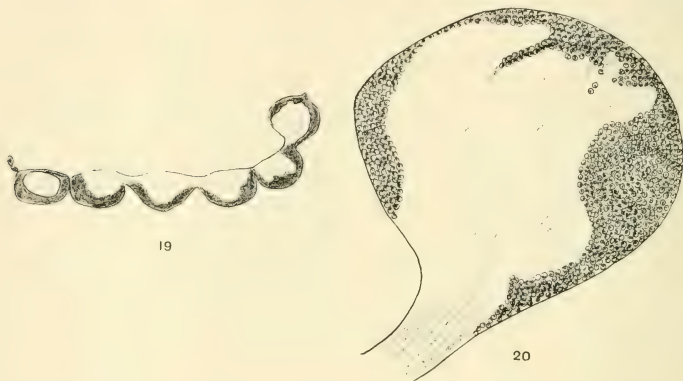


Fig. 19 A longitudinal section through the central ganglia of *Leecythorhynchus marginatus*, Cole. Two small abdominal ganglia show at the end of the last thoracic ganglion. $\times 35$.

Fig. 20 A longitudinal section through the supraesophageal ganglion of *L. marginatus*. The dorsal side is up, the cephalic side to the right. $\times 210$.

dominal. In a stage just before this there are two pairs of ganglia on the dorsal side of the larva; these are shown in figure 16. They represent the brain.

At about the third moult, as shown in figures 12 and 17, the ganglia have developed central fibers, but still show their paired nature. There seems to be some indication of more ganglia than there are appendages, some of the caudal elements may not be evident in later stages, and the first ventral ganglion seems composed of two small pairs of elements. In the proboscis

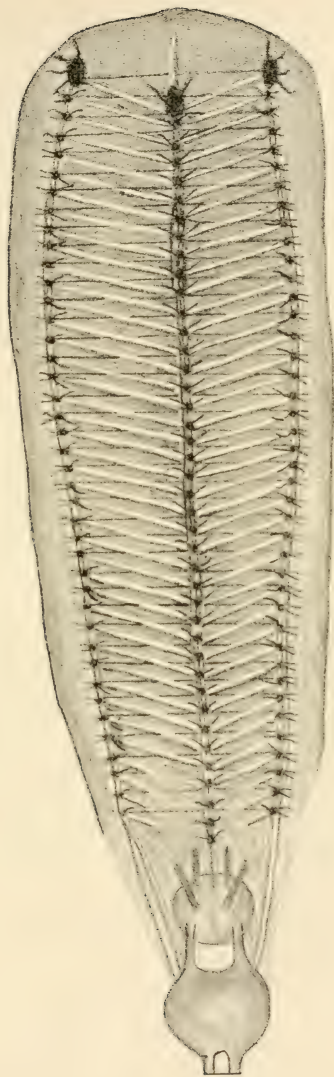


Fig. 21. Drawing of the nerves and ganglia of the proboscis of *L. marginatus*. Slightly diagrammatic. No structures shown in the proboscis but nerves and ganglia. The drawing was made by Miss M. L. Moles from the first sketch taken from the dissection. Much enlarged.

of this stage there seem to be two small pairs of ganglia. The dorsal ganglia are not shown in figure 17.

When the larvae moult again and leave the cavity of the hydroids they have all but one pair of legs. Figure 18 shows the whole central nervous system from below at such a stage. The brain above the esophagus is at the upper end of the figure, then follow the ventral ganglia, seven paired masses and a small unpaired caudal ganglion. There is a gradual fusion of these ganglia until the adult condition shown in figure 7 is attained.

The structure of the adult nervous system of pycnogonids is quite simple. There is the same general arrangement of cells that we find in other arthropods. The ventral ganglia have few cells on the dorsal side, but many on the lateral and ventral sides. The supraesophageal ganglion is sheathed in cells on the lateral and dorsal sides. Nerve fibers connect the ganglia and certain regions but in no place is there a concentration of the fibers. The fibrous mass is not particularly dense at any point. There do not seem to be many long tracts and the supraesophageal ganglion is not more complicated than other parts so far as could be determined. There are no marked decussations of nerve fibers and the nerve cells present a uniform appearance. Among the nerve cells are many nuclei of neuroglia networks which form the framework of the ganglia especially in the area of the cells.

Although there are indications of special groups of cells and fibers, there was no indications of mushroom bodies.

The animals do not seem to have a special brain. The supraesophageal ganglion is not a very special center. The movements of the animals agree with this; they move sideways, forwards or backwards when stimulated. No part of the body seems to lead in the locomotion.

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EVIDENCE OF A MOTOR PALLIUM IN THE FORE-BRAIN OF REPTILES¹

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ONE FIGURE

At the title indicates, the purpose of this note is not to describe the structure of the reptilian motor cortex nor to discuss the localization of function within this area. The purpose is only to give such evidence as is now at hand that a specialized area comparable to the mammalian motor cortex probably exists in the reptilian brain and to indicate the general position and extent of this area.

The writer has indicated in a previous paper ('15 b) that the rostral portion of the dorsal pallium in the turtle differs structurally from the rest and has suggested the possibility that this area may correspond to the motor cortical area in the mammalian brain.

This hypothesis is being tested by degeneration methods and by cortical stimulation. Sufficiently definite results have been obtained by the latter method to indicate that motor and sensory fields can be distinguished in the reptilian pallium.

Three species of turtle (*Chelydra serpentina*, *Cistudo carolina*, and *Chrysemys marginata*) and one lizard (*Gerrhonotus*) have been studied. Two methods of stimulation were used: induction shocks with two-point electrodes, the two points closely approximated; induction shocks with one point electrode, the second electrode being formed by a copper plate covered with moist cloth on which the body of the animal rested.

An important factor in the experiments is the degree of anaesthesia employed. The clearest results have been obtained with very deep anaesthesia. Some of the turtles were given a dose of morphine by hypodermic injection, and all were anaesthet-

¹ Neurological Studies, University of Minnesota, no. 22, June 29, 1916.

ized by means of chloroform placed on cotton in the mouth or injected directly into the trachea or both. Although the resistance of these animals was well known, in several cases the anaesthesia was not carried far enough and the reactions to cortical stimulation were prolonged contractions of neck muscles or struggling movements of the limbs indicative of pain. In these cases occasional responses, as of the eye muscles or temporal muscle, appeared to be due to local excitation of the cortex but the more extensive and prolonged movements were elicited from any part of the cortex and were not distinguishable from the responses to stimulation of the dura mater, the lower brain regions or even the tissues exposed in the head. Under conditions of deep anaesthesia the responses consisted of contraction of a small set of muscles, and of short duration, and these were obtained from a certain region of the pallium only. The lizards are not so difficult to anaesthetize, but long continued application of chloroform is necessary (one and a half to two hours in a closed dish containing a wad of cotton wet in chloroform).

Another factor presenting difficulty is the small size of the brain in these animals. This makes it necessary to use a single point electrode or to have the two points very close together. It is of the greatest importance that the meninges be carefully removed from the whole hemisphere and that the brain and surrounding tissues be kept as dry as possible without injury to the brain tissue. If the electrode comes too close to the dura mater or if fluid facilitates the spread of the current to the dura, the muscles, thalamus or midbrain, the results are vitiated because of responses coming from two or more sources of stimulation. The responses which follow stimulation of the dura or brain stem are very different from those produced by cortical excitation, being usually more vigorous and prolonged as well as more extensive. In the lizards and in several turtles, after the dura was laid back and the olfactory and optic connections were cut, the entire forebrain was raised out of the skull so as to be free from contact with other tissues. Under these conditions the entire surface of the hemisphere was explored with the electrodes.

With the induced current minimal stimuli were applied at first and increased later in the experiment when it became necessary in order to obtain responses. In many cases the brain fatigued quickly and in some it recovered with equal promptness. Individuals differed greatly as to the recovery. Often when the strength of stimulus was increased struggling movements resulted and the experiment had to be discontinued. In some cases also, the animals gave definitely localized responses to the first stimulation and later, probably because of recovery from the anaesthesia, gave irregular and prolonged reactions.

CORTICAL REACTIONS IN TURTLES

If we take into account only the short contraction of restricted groups of muscles, the following parts of the hemisphere have been found to give muscular responses: dorsal surface of the olfactory bulb, retraction of the neck, extension of the legs, movements of eyeball and eyelid; dorsal surface of pallium near olfactory peduncle and lateral border of pallium in the anterior one-half or two-thirds of hemisphere, movements of eyes, jaw, neck, legs and tail; striatal area, movements of all parts. No responses were obtained from any other part of the dorsal surface or the medial wall or from the tuberculum olfactorium, the amygdaloid eminence or elsewhere, except that contractions typical of thalamic stimulation were sometimes obtained when the caudal pole or amygdaloid eminence was being explored. These were probably due to spread of current to the thalamus.

The responses from the striatal area were presumably due to the direct stimulation of descending fibers in the crus. The responses from the olfactory bulb may be due to the close proximity of the 'motor area' which is indeed overlapped by the caudal border of the olfactory formation. Thus it appears that a somewhat comma-shaped area involving the rostral and lateral borders of the general pallium (fig. 1) may be regarded as a motor area in the turtle's pallium. This area corresponds roughly to the 'pallial thickening' described in a previous paper ('15 b).

Not enough experiments have been conducted under uniform conditions to furnish a basis for the discussion of localization

within this area. As the whole area is only a few millimeters in extent, it is obvious that great care must be exercised in accepting as conclusive any apparent indications of special function of particular parts of this area. For this reason I mention only tentatively and with reserve that leg movements have been obtained most often from the anterior part of this area and eye, jaw and neck movements from the lateral portion. Further experiments will be made to determine whether a localization within the motor area clearly exists. In certain experiments

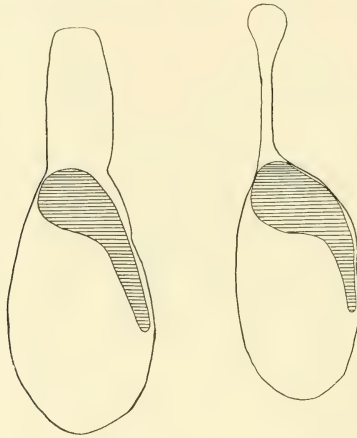


Fig. 1 Sketches of the dorsal surface of the right hemisphere of the turtle (left) and lizard (right) on which the motor area is shaded.

the eye and jaw movements were homolateral only or more frequently, while the neck and leg movements were mostly heterolateral.

Excitation of the dorsal ventricular ridge in the turtle was carried out by cutting away the whole dorsal pallium and applying the electrodes to the ventricular surface of the ridge. Muscular responses were often obtained, such as retraction of the eyeball, contraction of temporal muscle and twitching of fore leg, but there is the double danger of spread of stimulus to the closely adjacent pallial thickening and of stimulation of the underlying crus.

MOTOR RESPONSES IN THE LIZARD

Only four animals have been examined, two of which gave much clearer and more definite results than those obtained from most of the turtles. From the area shown in the accompanying sketch one animal gave movements of the fore leg, jaw and eye muscles, neck and throat muscles, and anterior body musculature. Stimulation of the olfactory tubercle also caused contraction of small muscles in the throat, which was felt as the head was held in the fingers. Stimulation of the crus in the striatal area produced movements of legs and body muscles. Movements of the fore leg were regularly heterolateral, although homolateral movements occurred occasionally. Just the opposite was true of the eye and jaw muscles. The other animal responded to stimulation of the anterior part of this area by strong contraction of pelvic muscles and movements of the hind legs and by weak movements of the fore legs. Stimulation of the lateral border of the right pallium in this animal produced a definite torsion of the fore part of the body.

Gerrhonotus is a rather large lizard and its brain is fairly satisfactory to work with. The results in these two cases were so definite that I regard them as strong evidence that a motor area exists in the lizard pallium essentially similar in position and extent to that in the turtle.

Early in my studies I examined the brain of a single specimen of *Alligator* about a foot and a half in length, and obtained motor responses from the anterior part of the dorsal pallium, but I have no written record of this experiment.

From these experiments, together with the study of the structure of the hemisphere already reported, I am strongly inclined to believe not only that reptiles possess a general or somatic pallium which has been in dispute until recently, but also that in that pallium are to be distinguished definite sensory and motor areas in the sense in which those terms are commonly used of the mammalian pallium. Localization within these areas and the significance of these areas in the evolution of the mammalian pallium are subjects for further study.

THE DEVELOPMENT OF THE DORSAL VENTRICULAR RIDGE IN TURTLES¹

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TWENTY-SEVEN FIGURES

In the adult turtle (Johnston, '15 b) the large ridge projecting into the ventricle from the lateral wall of the hemisphere has strong fiber connections with the cerebral peduncle and has obvious close relations with the cortical layers of the pallium in the caudal pole. Indeed, in its caudal part this ridge appears to be a fold of the whole thickness of the brain wall and is marked externally by the 'amygdaloid fissure.' Here the cell layers of the general pallium turn in to become continuous with the cell masses of the ridge. It is evident from these facts that the relations of the dorsal ventricular ridge are chiefly with the general pallium. Its position lateral and dorsal to both the caudate and lentiform nuclei shows that it does not belong to the corpus striatum. Its independence from other cell masses in its rostral part gives one the impression (from the study of adult material alone), that the ridge has been formed by infolding from the caudal region ('15 b, p. 417).

The origin of this ridge in the embryo should throw light not only on its relations in the turtle but also on its significance in the evolution of the mammalian brain. I am able to give the main outlines of the development on the basis of a series of early embryos of *Chelydra serpentina*, for which I am indebted to Dr. C. E. Johnson of this University; and of sections of 17, 20, and 28 mm. embryos of *Chrysemys*, belonging to the Anatomical Laboratory of Washington University for the loan of which I am indebted to Dr. Edwin A. Baumgartner.

¹ Neurological Studies, University of Minnesota. No. 23, July 1, 1916.

The material in both cases was beautifully sectioned and stained. The brains of freshwater turtles are so similar that the younger stages of *Chelydra* can be readily compared with the later stages of *Chrysemys* for the rather general purposes of this study.

AREAS OF PROLIFERATION IN THE EARLY EMBRYO IN RELATION
TO CELL MASSES OF THE ADULT BRAIN

Broadly speaking, three periods are to be recognized in the development of cell masses in the telencephalon: a period of evagination of the hemispheres and formation of indifferent cells by mitosis of the germinal cells; a period of form changes and further proliferation of cells in which the successive formation of functionally independent cell masses is noticeable; and a period during which the growth of fiber bundles and other factors bring about the definitive form of the adult cell masses.

In the first period the wall of the telencephalon consists of a deep layer of spongioblasts and dividing germinal cells, a thick layer of indifferent cells and a superficial clear layer or marginal veil of very varying thickness. The thickest part of the marginal veil forms the floor of the groove along the lower border of the hemisphere, at its junction with the brain stem. This is the course of the future lateral forebrain bundle or *crus cerebri*. The indifferent cells form a continuous layer with no indication of individual masses.

The second period begins with the appearance of independent cell masses separated by cell-free zones which show in sections as clear lines or areas. Such cell-free zones in adult brains have been commonly regarded as boundary lines between individual cell masses which are presumed to have different functions; i.e., centers or nuclei. Similar cell-free zones in the brains of lower vertebrates have been used in recent years as landmarks between important morphological regions, as the pallial and basal areas. The writer has pointed out the need of caution in interpreting these zones in this connection ('13 b, p. 382). It is now to be noted that the formation of individual masses of cells is dependent upon the proliferation of cells from different regions of the germinal cell layer and upon the proliferation

of cells at successive periods of development. Thus the proliferation of cells in the dorsal wall gives rise to pallium, that in the basal wall gives rise to various olfactory centers, corpus striatum, etc. Also, certain centers owe their origin to the proliferation of cells in an early stage, other centers to proliferation in later stages. The former come to lie superficial to the latter and the two are separated by cell-free zones. A further factor is the shifting or spreading of cell masses, whether due to cell-migration or to mechanical forces. These several factors must be taken into account in any attempt to use the embryological method in the study of the significance of cell-grouping in the brain.

CHELYDRA SERPENTINA

In the oldest *Chelydra* embryo studied, it is possible to recognize several of the important cell masses and fiber bundles of the telencephalon and in part the boundary lines between pallial and basal areas. Examination of the model (figs. 1, 2) and of sections (figs. 6 to 14) shows that the telencephalon and diencephalon are narrow from side to side and high dorso-ventrally. The hemisphere is a simple sac which projects much farther rostrad than caudad. The interventricular foramen is still very large. Behind it, the thin wall which represents the choroid plexus extends nearly half way to the caudal pole. This stage illustrates very clearly, both in its general form and its internal structure, how much the rostral pole of the hemisphere precedes the caudal pole in development. The outer surface of the brain shows few of the landmarks which are seen in later embryos and adults. The stem-hemisphere sulcus is of course the most prominent. The olfactory peduncle is not yet formed, but the broad depression on the lateral surface may represent the beginning of constriction. The fissura prima and diagonal band are recognizable at the medio-basal angle in front of the preoptic recess. There is a shallow furrow over the rostral part of the foramen which may be the beginning of the sulcus fimbrio-dentatus.

The ventricle shows a very sharp ventral groove leading forward from the foramen and much deepened in the region of the future tuberculum olfactorium. The middle ventricular groove is clearly marked in the region rostral to the foramen, while the dorsal groove has not yet appeared.

In spite of the simple form of this brain, the internal structure shows considerable advance in differentiation.

The sections illustrated in figures 6 to 14 can be understood best by beginning with that which passes through the rostral border of the foramen interventriculare (fig. 9). In the medial wall above the foramen are recognized the primordium hippocampi and the fimbria, one of the early fiber tracts to be formed in the embryo. The dorsal wall is occupied by pallium, the medial portion by hippocampal, the lateral portion by general pallium. In the lateral wall the pallium seems to be limited by a ventricular groove and by a cell-free space. This boundary line strongly reminds one of the lateral zona limitans of the selachian and frog brains (Johnston, '11a). The later embryos show this groove to be the middle ventricular groove of the adult brain and the clear area becomes the cell-free zone separating the dorsal ventricular ridge and the nucleus lentiformis in the adult. In the lower half of the lateral wall the following structures are seen in the figure: an active proliferation which gives rise to the nucleus lentiformis, the nucleus caudatus, the lateral and medial forebrain bundles, the anterior commissure bundle, the fiber layer of the diagonal band, and the lateral olfactory area. Although I have not sufficient stages to enable me to follow the history of these structures, there is evidence that the superficial cell layer which represents the lateral olfactory nucleus has been derived from the layers of indifferent cells in the early embryo and that the mass of the caudate nucleus represents a later proliferation. The embryo shows clearly that the lentiform nucleus is beginning to form when the caudate is already completely formed or nearly so. As the other sections are studied it will become evident that the lentiform nucleus is formed by a proliferation distinctly dorsal to the caudate and that the changes in later development bring

it into a position lateral to the caudate. There is some indication of the superficial layer of the olfactory area spreading upward beyond the middle ventricular groove (at the point marked *l.pyr*).

Passing caudad from this level, in the next section drawn (fig. 8) the same structures are to be seen in the lateral wall. The middle ventricular groove has almost disappeared, however, and the proliferating area for the lentiform nucleus is much smaller. The next section drawn (fig. 7) is the last one in which the lentiform proliferation can be recognized and with it the locus of the pallial border. In the lower part of the hemisphere the lateral olfactory area is represented by a large collection of cells closely related to the caudate nucleus and the diagonal band. This is the nucleus of the lateral olfactory tract and the next section shows the close relation of both this and the diagonal band to the stria medullaris. Caudal to this the nucleus of the lateral olfactory tract soon disappears and the hemisphere wall presents the appearance of an undifferentiated pallium, except for the fimbria and the thin choroidal area in the medial wall.

The next section rostrad from the one first described passes through the anterior commissure (fig. 10). The lentiform proliferation appears larger, the diagonal band fibers approach those of the olfacto-hypothalamic system. In figures 9, 10, and those of sections farther rostrad, one of the most noteworthy features is the mass of cells proliferating from the deep layers of the pallium. The medial border of the pallium does not show active proliferation but in the lateral three-fourths many new cells are forming and these appear to be streaming laterad into the thick wall just dorsal to the middle ventricular groove. Indeed the proliferation and streaming of these cells is the active cause for the thickening of this part of the wall and the formation of the middle ventricular groove. Further thickening of this part of the wall deepens this groove and produces the dorsal ventricular groove at the point indicated in figure 9.

In figures 9 to 12 appear important relations of the dorsal border of the olfactory area to the pallium. Here it is quite

clear that the superficial layer of cells of the olfactory area extends up on the outer surface of the pallium and that it is accompanied by a special bundle of fibers. The crowding of cells in this stage is such that it is impossible to determine whether the pyriform lobe cells have spread or migrated up from the lateral surface in the basal region or have been formed here by the proliferation which at this stage is giving rise to the pallium. Farther caudad and in later stages the relations are such as to make the former appear more probable. The cells of the pyriform lobe probably all come from the layer of indifferent cells seen in earlier stages and formed by proliferation in the lateral and basal region before the pallial proliferation seen in this stage began. Pallium and pyriform lobe are still visible in a section (fig. 14) through the peduncle in which the olfactory formation appears in the dorsal wall. The olfactory formation extends far back on the dorsal surface, as is already well known.

CHRYSEMYS

Models were made of the 17 mm. and 28 mm. stages. Most of the chief landmarks of the adult brain are already visible in the 17 mm. embryo (figs. 3, 4, 15, 16, 17). The caudal pole still lags behind the rostral portion in development. The caudal pole projects much farther back than in the oldest *Chelydra* embryo and in the 28 mm. stage the olfactory area (lobus pyriformis) extends relatively farther back than in the 17 mm. stage. The tuberculum olfactorium is well developed and the ventral groove of the ventricle dips deep into it. The middle ventricular groove (fig. 4 and sections) is very sharp and deep in the middle part of the brain and stops abruptly just caudal to the level of the foramen interventriculare. The dorsal ventricular groove appears in the 17 mm. embryo (figs. 4, 17) as a slight groove in the lateral wall some distance from the medio-dorsal angle of the ventricle.

Three sections caudal to the foramen are drawn from the 17 mm. embryo and nine sections from various levels of the 28 mm. embryo. The internal differentiation has already progressed so far in the 17 mm. embryo that it will be unnecessary

to speak of it separately. In both stages many features of the sections can readily be compared with the structures seen in corresponding sections of the adult brain. For this purpose the reader should consult the description of the cell masses in the brain of *Cistudo* in this Journal for October, 1915. The necessary description of individual sections is given in connection with the figures. Here I shall take up the relationships of the lateral margin of the pallium and the dorsal ventricular ridge.

Corpus striatum and lateral olfactory area. In the younger embryos the loosely arranged cells of the lateral olfactory centers cover the outer surface of the caudate and lentiform nuclei and form a continuous layer over the lateral surface of the hemisphere (except dorso-caudally). This is shown in figures 7 to 12, and this interpretation is given to the models. In the 28 mm. embryo there is a small area opposite the foramen in which the striatum and the crus bundle contained in it come to the surface (fig. 21) and the olfactory centers form the pyriform lobe above and the diagonal band below. This small area is painted blue in the model and appears white in figure 5. Sections show that it is the elbow of the crus, with certain large cells contained in it ('15 b, p. 405), which comes to the surface here. This is presumably due to the rapid growth of both ascending and descending fibers in the crus. The crowding thus produced leads to the shifting of the cells of the olfactory center upward and downward. This process must go much further in later development until the large striatal area is left exposed in the adult. The embryonic history thus seems to support very clearly the hypothesis ('15 b, p. 428, 429) that the exposed striatal area in the turtle had been covered at an earlier stage of evolution by the lateral olfactory area.

Relations of pyriform lobe and pallial margin. In the caudal half of the hemisphere the pyriform lobe forms a layer external to the corpus striatum and extends up to a thin edge superficial to the dorsal ventricular ridge and the margin of the pallium. This overlapping of the pallial margin by the pyriform lobe persists in the adult. At about the level of the interventricular

foramen the pyriform lobe begins to extend higher on the lateral surface. At the same time thickening of the lateral border of the pallium moves dorsally, becomes crowded and folded on itself and sinks in, forming a projection into the ventricle (figs. 22 to 25). These changes take place rapidly from behind forward so that in thirty or forty sections of 10 microns the place occupied by the thick border of the pallium comes to be taken by the pyriform lobe. From this point forward the pyriform lobe forms a characteristic ridge filled with a dense layer of cells, very much as in the adult.

Origin of dorsal ventricular ridge. In the *Chelydra* embryo above described the dorsal part of the lateral wall is thickened by cells coming from a proliferating area in the dorso-lateral wall. The groove which bounds this thickening was identified with the middle ventricular groove of the adult. In the older embryos of *Chysemys* the groove is readily identified but has been very greatly deepened by further thickening of the mass just above it. Likewise the thickening of this mass has produced a groove above it, the dorsal ventricular groove. The thick mass, then, is the dorsal ventricular ridge and in earlier stages it is indistinguishable from the dorsal pallium. In the caudal part of the hemisphere of the older embryos the relations of the dorsal ventricular ridge are not essentially changed from those seen in the *Chelydra* embryo. Caudal to the level of the stria medullaris (figs. 18, 19) this ridge bulges into the ventricle and is covered outside and below by the thick mass of the nucleus of the lateral olfactory tract. In the caudal pole the ridge merges gradually with the general pallium caudal to the level at which the pyriform lobe ends. In the rostral part of the hemisphere (figs. 22, 23) the dorsal ridge has become practically independent of the pallial thickening, very much as it is in the adult. From these embryos it is quite clear that the dorsal ventricular ridge arises as a thickening of the lateral border of the pallium and that its continued growth causes it to project into the ventricle and produces the middle and dorsal ventricular grooves. In the 28 mm. embryo the mass of cells which in the adult was called the core-nucleus of the ridge

is clearly recognizable in the rostral part, back to the level of the caudal border of the foramen interventriculare.

Between the pallial thickening and the cells of the dorsal ventricular ridge appears in the sections a clear space filled by fibers. In the caudal part of the hemisphere, where the dorsal ventricular groove is already formed, this bundle of fibers is seen to hold the same position as an important bundle in the adult. In the adult many fibers from the crus enter this bundle or, more probably, pass through it on their way to the pallium. Here in the embryo the crus is not yet sufficiently developed to enable one to trace it up through the striatum to this level. The strong development at this stage of the bundle here mentioned indicates that it is composed chiefly of fibers connecting parts of the hemisphere; an association bundle, in other words.

Relations of dorsal ventricular ridge to basal structures. In the rostral part of the hemisphere the ridge is from the first clearly marked off from the striatum below by the middle groove and by a prominent cell-free zone. In later embryos this cell-free zone becomes compressed into a narrower line as seen in section and scattering cells are found in it. There is, however, always a clear boundary between the ridge and the developing lentiform nucleus. In section the cell-free zone inclines dorsad toward the lateral surface and passes above the lateral olfactory area. In the rostral part, where the pyriform lobe crowds far dorsal this limiting zone is bent into a semicircular or U-shaped line (figs. 23 to 27). When the writer first studied the adult turtle brain he found it impossible to establish a direct comparison between the lateral zona limitans of the selachian, frog and other simpler vertebrates, and either of the cell-free zones seen in the lateral wall of the turtle brain. It is now evident that the clear zone which in the adult brain passes from a point near the middle ventricular groove toward the lateral surface and then bends dorsad to reach the surface in the sulcus rhinalis above the lobus pyriformis, is the zona limitans of the embryo and corresponds to the zona limitans lateralis in the selachian and frog. (See Johnston, '15 b, figs. 19 to 22 and compare with '11 a, fig. 75). Since the relations of the pyri-

form lobe, corpus striatum and pallium in the turtle are readily comparable with those in the mammal, the identification of the lateral zona limitans in the turtle completes the history of this important landmark throughout the series of vertebrates. The boundary lines between the pallial and basal portions of the hemisphere are now clear in both medial and lateral walls in fishes, amphibians, reptiles and mammals (Johnston, '11 a, '13 b, '15 b).

The relation of the dorsal ventricular ridge to the amygdaloid complex requires further comparative study. In the adult, it will be remembered, the caudal part of this ridge is separated from the rest by a broad shallow groove which continues in the general direction of the deep middle ventricular groove ('15 b, figs. 4 and 10). This caudal portion of the ridge is made up of the medial amygdaloid nucleus and of an apparent infolding of the general pallium. Even in the oldest of these embryos the dorsal ventricular ridge extends into the tip of the caudal pole as a simple thickening of the general pallium. It seems probable, although the evidence is not sufficient for a definite conclusion, that the caudal portion of the ridge including the large-celled medial amygdaloid nucleus is formed from this thickening of general pallium.

For the photographs of the models I am indebted to Dr. W. F. Allen of the University of Oregon.

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FIGURES

ABBREVIATIONS

<i>a.p.</i> , area parolfactoria	<i>n.c.</i> , nucleus caudatus
<i>b.o.</i> , bulbus olfactorius	<i>n.d.b.</i> , nucleus of the diagonal band
<i>c.a.</i> , commissura anterior	<i>n.l.</i> , nucleus lentiformis
<i>c.h.</i> , commissura hippocampi	<i>n.o.</i> , nervus olfactorius
<i>ch. op.</i> , chiasma opticum	<i>n.rot.</i> , nucleus rotundus
<i>c.p.a.</i> , commissura pallii anterior	<i>n.tr.olf.lat.</i> , nucleus of the lateral olfactory tract
<i>crus</i> , crus cerebri or lateral forebrain bundle	<i>pa.</i> , pallium
<i>c.st.</i> , corpus striatum	<i>pa.th.</i> , pallial thickening
<i>d.b.</i> , diagonal band of Broca	<i>p.h.</i> , primordium hippocampi
<i>d.v.r.</i> , dorsal ventricular ridge	<i>p.o.</i> , pedunculus olfactorius
<i>fi.</i> , fimbria	<i>r.p.</i> , recessus praecopticus
<i>f.o.</i> , formatio olfactoria	<i>r.s.</i> , recessus superior
<i>f.p.</i> , fissura prima	<i>s.f-d.</i> , sulcus fimbrio-dentatus
<i>for.i.</i> , foramen interventriculare	<i>s.m.</i> , stria medullaris
<i>f.rh.</i> , fissura rhinalis	<i>s.v.d.</i> , dorsal ventricular sulcus
<i>g.p.</i> , general pallium	<i>s.v.m.</i> , middle ventricular sulcus
<i>g.s.</i> , gyrus subcallosus	<i>s.v.v.</i> , ventral ventricular sulcus
<i>h.</i> , hippocampus	<i>thal.</i> , thalamus
<i>hy.</i> , hypothalamus	<i>t.o.</i> , tuberculum olfactorium
<i>l. pyr.</i> , lobus pyriformis	<i>tr.olf-hy.</i> , tractus olfacto-hypothalamicus
<i>l.t.</i> , lamina terminalis	<i>tr.op.</i> , tractus opticus
<i>m.fb.bdl.</i> , medial forebrain bundle	

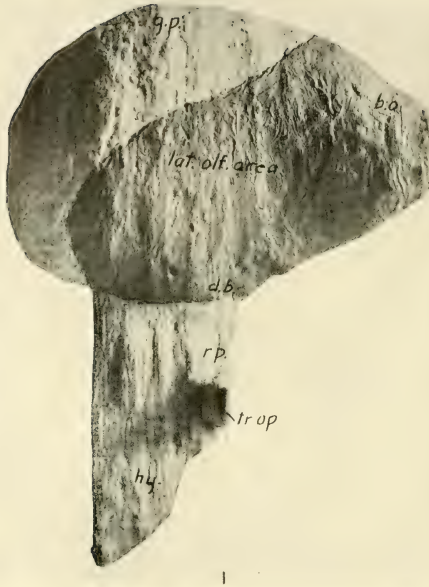
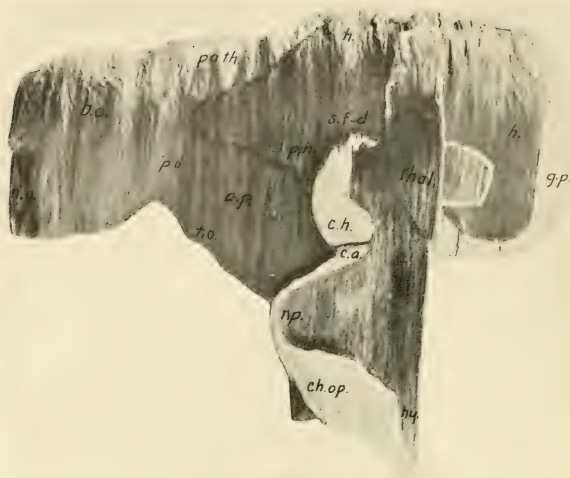


Fig. 1 *Chelydra serpentina*. The size of this embryo is indicated by the owner by the words '8.5 mm. carapace.' Lateral view of a model of the right hemisphere. Description in the text.

This and the following models have been merely rubbed with a blunt instrument to smooth the surface. The external surface has been painted so that the olfactory centers and hippocampus appear dark and the general pallium light. The diencephalon has been left with the gray color of the modelling paper and the ventricular surface of the hemisphere has had no treatment whatever. The lamina terminalis and line of attachment of the choroid plexus have been painted white. All the models were made at a magnification of 50 diameters and are reduced one-third in reproduction.



Fig. 2 Medial view of the model shown in figure 1. Description in the text



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Fig. 3 *Chrysemys* embryo of 17 mm. Medial view of a model of the right hemisphere. The narrowing of the interventricular foramen seems to have taken place from above downward on account of the expansion of the hippocampus and general pallium. The white area labelled *c.h.*, is the cut surface of the primordium hippocampi in the median plane. The thin portion of the medial hemisphere wall which will form the choroid fissure and plexus is bounded by a white line.

Fig. 4 Same model as figure 3, with the thalamus, hippocampus and primordium hippocampi removed. The lateral wall of the ventricle shows the dorsal ridge bounded below by the deep middle ventricular groove as described in the text. The dorsal groove is shallow. There is no evident boundary between the dorsal ridge and the pallial thickening at the rostral end.

Fig. 5 *Chrysemys*, 28 mm. Lateral view of a model of the right hemisphere. Note the great elongation of the pyriform lobe in the caudal pole as compared with the condition in figure 1. With regard to the exposure of the corpus striatum on the lateral surface, compare figures 1 and 5 with the figures in '15 b.

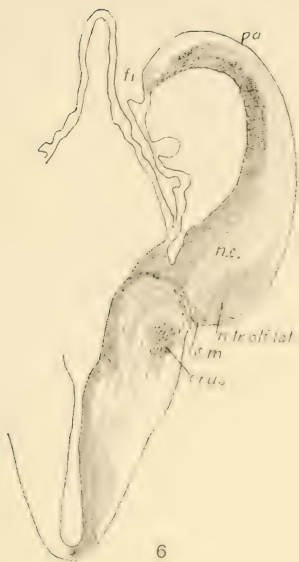
Figs. 6 to 14 Transverse sections through the right hemisphere of the Chelydra embryo illustrated in figures 1 and 2.

Fig. 6 Section just caudal to the foramen interventriculare. This is the most caudal section in which the nucleus caudatus can be recognized and here it practically fuses with the nucleus of the lateral olfactory tract. Both are undoubtedly parts of the primitive basal olfactory centers which have been carried out into the caudal pole of the hemisphere evagination. There is in this section no apparent boundary between these nuclei which enter into the amygdaloid complex and the general pallium.

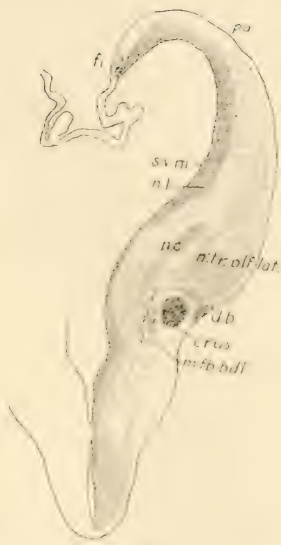
Fig. 7 Section through the caudal part of the foramen, 120 microns rostral to figure 6. The nucleus caudatus is much more distinct and the proliferation of cells for the nucleus lentiformis begins to appear. There is now a slight indication of the lateral boundary of the pallium. The letters *s.v.m.* in this and figure 8 indicate the position in which the middle ventricular sulcus is to be formed, although it is not actually present in these sections.

Fig. 8 Section through rostral part of the foramen, 100 microns rostral to figure 7. The lateral olfactory area is represented chiefly by cell masses adjacent to the diagonal band. The nucleus caudatus begins to be separated from this area by the crus entering the hemisphere. The lentiform proliferation is larger and the clear space just above it, which corresponds to the zona limitans lateralis in lower vertebrates, is present from this level forward.

Fig. 9 Section through the rostral wall of the foramen. The position of the foramen is represented by the light space at the medio-ventral angle of the ventricle. The crus has now separated the nucleus caudatus from the nucleus of the diagonal band and both are somewhat removed from the larger mass of the lateral olfactory area which is here labeled *l.pyr.* The middle ventricular sulcus which is present in this section extends farther caudally in older embryos and adult.



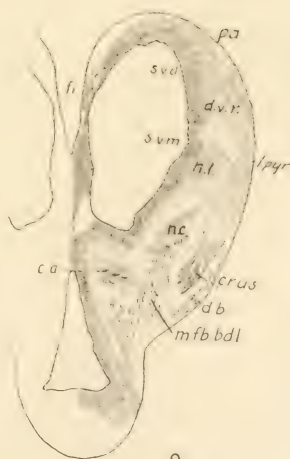
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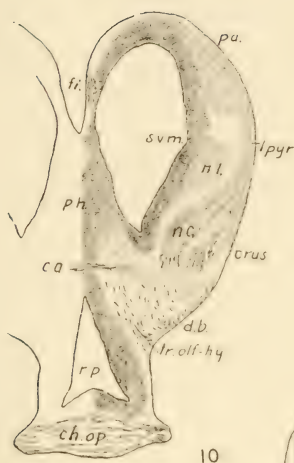
Fig. 10 Section through the anterior commissure. Although the lateral olfactory area is continuous in these sections and even the nucleus caudatus is nowhere wholly separated from it, one can clearly see the beginnings of the segregation of the pyriform lobe, diagonal band and caudate nucleus by reason of the enlargement of the lateral forebrain bundle (*crus*). This process will be completed in later stages by the development of the lentiform nucleus and the further enlargement of the *crus*.

Fig. 11 Section just rostral to the preoptic recess where the diagonal band turns up in the medial wall. The dark mass on the medial surface of the area parolfactoria represents the beginning of the nucleus of the diagonal band, which corresponds to the gyrus subcallosus of the mammalian brain. The nucleus caudatus is connected beneath the ventricle with the area parolfactoria. From this level rostrad the olfactory area or lobe is quite continuous from its lateral border at *l.pyr.* to its medial border near *fi*.

Fig. 12 Section 60 microns rostral to figure 11, where the diagonal band fibers rise up in the medial wall to join the fimbria. Although the cells of the pyriform lobe and pallium lie immediately in contact, there appears in many sections a very sharp line as if it were caused by a delicate septum running dorsad from the small fiber bundle beneath *l.pyr.*

Fig. 13 Section through the tuberculum olfactorium. Note that the ventral ventricular sulcus here descends nearer to the surface than elsewhere so that the somewhat bulging tuberculum contains a large pouch of the ventricle.

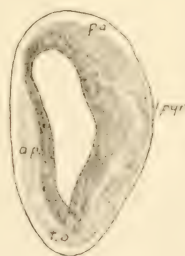
Fig. 14 Section through the olfactory peduncle. The ventral part of the section cuts the rostral wall of the tuberculum while its dorsal part cuts the olfactory formation.



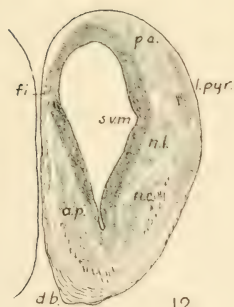
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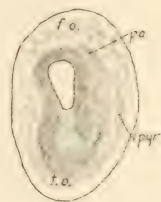
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Figs. 15, 16, 17 Three transverse sections of the right hemisphere of the 17 mm. *Chrysemys*. Figure 17 falls in the caudal part of the foramen and figure 15 represents next to the last section in which the connection of the hemisphere with the thalamus is seen. Figure 16 is between these two.

In this embryo the mass formed by the pallial proliferation of the earlier stage has become divided into a general pallium and the dorsal ventricular ridge. The ridge projects into the ventricle forming a deep middle ventricular sulcus and a shallow dorsal sulcus. Note that the dorsal sulcus is quite independent of the dorso-medial angle of the ventricle. The dorsal ventricular ridge therefore is clearly a thickening of the lateral wall above the lateral zona limitans. This zona limitans is the light streak in the drawings running diagonally between *d.v.r.* above and *n.l.* and *l.pyr.* below. The nucleus of the diagonal band is very large and definite in figure 17, while in figure 15 and 16 its substance is fused with the nucleus of the lateral olfactory tract. The lentiform proliferation is still in progress, especially from the dorsal lip of the middle sulcus. The peculiar arrangement of ependyma cells seen at this point in the adult already begins to be apparent here.

Figs. 18 to 27 Transverse sections of the right hemisphere of the 28 mm. embryo of *Chrysemys*.

Fig. 18 Section at about the point where the fimbria curves down behind the choroid area. Caudal to this the nucleus of the lateral olfactory tract soon disappears and the dorsal ridge continues on into the caudal pole as a simple thickening of the lateral walls. At *g.p.?* is a partly segregated mass of cells which probably represents general pallium extending from the caudal pole forward to this point in the basal wall as it does in the adult.

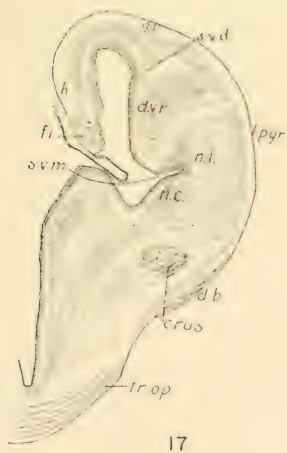
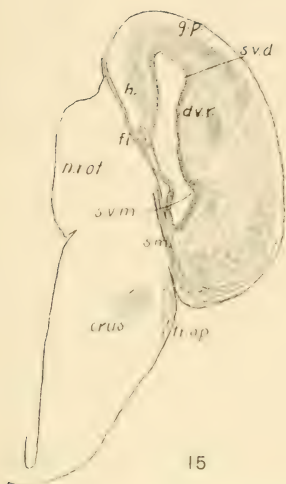
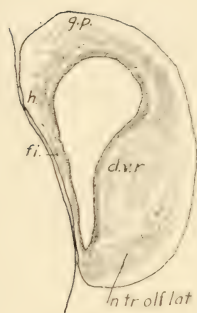


Fig. 19 Section 120 microns rostral to figure 18. In both these sections the connection of the dorsal ridge with the pallium is clear and they illustrate the more embryonic conditions which prevail in the caudal pole than in the rostral part of the hemisphere.

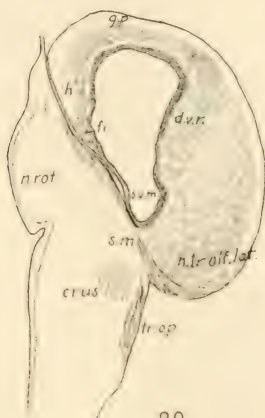
Fig. 20 Section at the point of connection of the hemisphere with the thalamus where the stria medullaris passes into the thalamus. In this and the next two figures the lateral zona limitans appears as a diagonal light line running dorsolaterally from *s.v.m.* to the outer surface.

Eig. 21 Section through the foramen interventriculare. The lentiform and caudate nuclei, neither of which was distinguishable in figure 20, are here quite distinct. The crus in this figure separates the caudate from the nucleus of the diagonal band. From this point rostrad for thirty to forty sections the crus lies practically exposed on the lateral surface. This condition is represented by a small striatal area in the model of this brain (fig. 5).

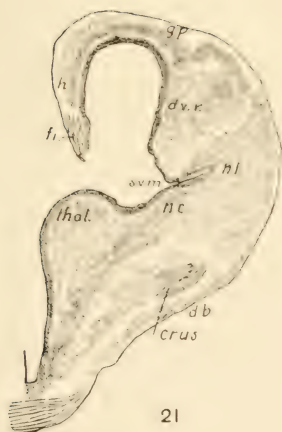
Fig. 22 Section through the anterior commissure. Here the general pallium begins to be decidedly thickened. The motor area of the adult includes this region and extends farther caudad. Forward from this level the olfactory area is again continuous on the lateral, basal and medial surfaces.



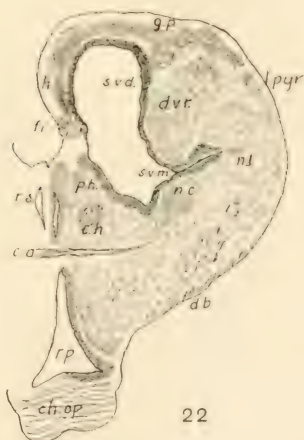
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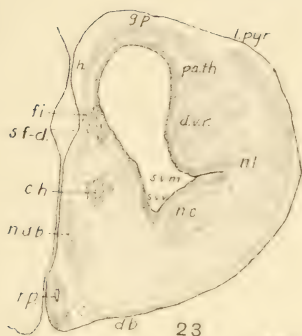


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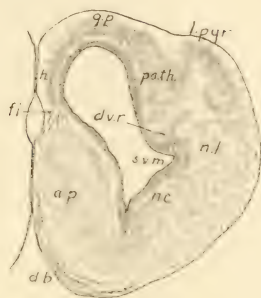
Fig. 23 Section through the rostral wall of the preoptic recess and the nucleus of the diagonal band (gyrus subcallosus). Between the level of figure 22 and this the pyriform lobe has rapidly pushed up over the outer surface of the pallial thickening as described in the text. The dorsal ridge is decreasing in size and the arrangement of its cells in this and in figure 22 suggests the core-nucleus of the adult. The dorsal ridge and the pallial thickening form a common large ridge, while in the adult they are separated by a groove (see '15 b, fig. 10).

Fig. 24 Section through the rostral end of the dorsal ridge and through the fissura prima, in which the fibers of the diagonal band are turning up into the medial wall. Both pallial thickening and pyriform lobe are massive.

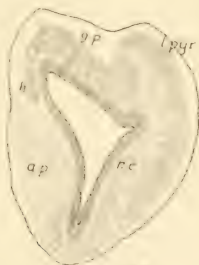
Figs. 25, 26 and 27 Sections through the anterior part or motor area of the general pallium. The whole of what is called general pallium here becomes more massive in the adult and is included in the pallial thickening. An important feature of these sections is that the general pallium especially at the dorso-medial angle is composed of conspicuously large cells. If the results of experiments reported elsewhere in this journal are to be credited, this is certainly a part of the motor area and probably is concerned with the control of the limbs. The presence of the large cells is readily understood if this is true.



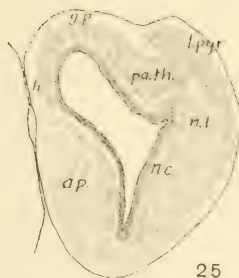
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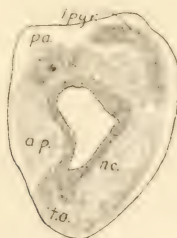
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THE MORPHOLOGY AND MORPHOGENESIS OF THE CHOROID PLEXUSES WITH ESPECIAL REFER- ENCE TO THE DEVELOPMENT OF THE LATERAL TELEENCEPHALIC PLEXUS IN CHRYSEMYS MARGINATA

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From the Anatomical Laboratory of the Northwestern University Medical School¹

TWENTY-SEVEN FIGURES

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INTRODUCTION

In a recent communication, the author (Bailey, '15) presented an interpretation of the lateral telencephalic choroid plexus in the human embryo based on ontogenetic and phylogenetic evidence. The phylogenetic evidence presented consisted of the most accurate descriptions available of the development of this structure in the lower vertebrates, viz., Warren's ('05) account of *Necturus maculatus* and Tandler and Kantor's ('07) account of *Platydaetylus mauritanicus*. With a view to confirming, and strengthening if possible, the phylogenetic evidence it was determined to investigate more definitely the development of the prosencephalic choroid plexuses in others of the lower vertebrates. The following scheme taken from Wilder was accepted

¹ Contribution No. 42. Submitted for publication July 20, 1916.

to represent as accurately as possible the phylogenetic stages of greatest value:

- Amphioxus
- Cyclostomes
- Selachians
- Ganoids
- Urodeles (for Stegocephali)
- Chelonia (for Theromorphs)
- Monotremes (for Pantatheria)
- Marsupials (for primitive Insectivora)
- Insectivora
- Lemurs (modern Mesodonta)
- Cercopithecoidae (tailed monkeys of Old World)
- Tailless Apes (Gorilla, etc.)
- Pithecanthropus (extinct)
- Homo primigenius (extinct)
- Homo sapiens

Below the Urodeles, the lateral telencephalic choroid plexus is very rudimentary if it appears at all. Its condition in the Urodeles has been thoroughly elucidated by Warren's ('05) account of *Necturus maculatus*. For the Chelonia, *Chrysemys marginata* was selected because it was readily accessible and also because a great part of the work had already been admirably done by Warren ('11) in the course of his investigation of the "Paraphysis and Pineal Region in Reptilia." It stands a little closer to the line of ascent of the mammals than the lizard, *Platydictylus mauritanicus*, described by Tandler and Kantor. Of the forms above the Chelonia, only *Didelphys virginiana* representing the Marsupials, was available. The present communication is concerned with *Chrysemys marginata* and a review of the literature, leaving *Didelphys* for a later paper.

HISTORY

There are no choroid plexuses in *Amphioxus* (Burekhardt, '94). Ahlborn ('83) describes well developed plexuses in the roof of the hindbrain of *Petromyzon planeri*, and also in the roof of the midbrain. The plexus of the diencephalic roof is not so well developed and no reference is made to any telencephalic plexuses. Burekhardt ('94) writes of *Petromyzon fluviatilis*:

Auch hier wie bei Teleostiern bleiben die Plexus inferioris (median telencephalic choroid plexus) nur in Gestalt einer Querfalte nachweisbar, die Paraphyse ist eine blosse Kuppel, der caudalen Rand wird als rudimentäres Velum auffassen.

There are no lateral telencephalic choroid plexuses in Cyclostomes.

Among the Selachians, however, there is a well formed plexus inferioris in *Notidanus* according to Burckhardt ('94), but no lateral telencephalic plexuses appear. On the other hand, I infer from Kappers and Carpenter ('11) that in *Chimaera monstrosa* the lateral plexuses are present.

Der ependymale Theil der Schlussplatte wölbt sich in ihrem frontalsten Abschnitt etwas über dem Niveau des Gehirnes hinaus eine Art paraphyse darstellend um sich dann plötzlich wieder einzufalten und in den unpaaren Ventrikel eindringend den plexus chorioideus ventriculi imparis zu bilden, wovon auch geringe Ausläufer in den schmalen Seitenventrikeln des Gehirns eindringen.

And Minot ('01) in describing *Acanthias* remarks:

The velum has now distinctly the character of a choroid plexus, being very irregular in the form of its surface, rich in blood vessels, covered by a thin ependyma and projecting far into the cavity of the brain. Laterally the projections from its surface are much more developed and as the organ has grown forward alongside the median paraphysal arch, it has produced what we can now easily identify as the plexus of the lateral ventricle. These plexuses are therefore to be interpreted morphologically as secondary modifications or appendages of the primary velum transversum. Attention should be paid to the two lateral projections, *L.ch.*, of the ependyma on the anterior surface of the velum, because these projections not only fix the lateral boundaries of the paraphysal arch but also are the anlagen of the choroid plexuses of the lateral ventricles. These anlagen from this stage (28.0 mm.) on rapidly increase both in size and in complication of form.

D'Erebia ('96) shows in *Torpedo* the velum transformed into a plexus and the tela chorioidea diencephali practically non-existent. In fact, this seems to be the tendency of these two structures in the entire Selachian group. There is a plexus in the roof of the fourth ventricle in all Selachians.

No plexus develops in the tela chorioidea telencephali medii in Ganoids, but in *Acipenser*, von Kupffer ('93) figures a plexus formation arising from the anterior wall of the velum trans-

versum, and Terry ('10) describes such a formation in *Amia*, as well as folds which appear on the caudal wall of the velum. The tela chorioidea diencephali itself forms a large thin-walled dome with no plexus formation. There is a plexus in the roof of the fourth ventricle. Lateral telencephalic choroid plexuses according to Burekhardt ('94) are absent, but Hill ('94) writing of *Amia* says: "It (the paraphysis) may be thought of as an isolated portion of the roof of the fore-brain which owes its existence to the formation of the folds marked *Pl.chr.* in figure 20, and which are themselves the representatives of the choroid plexuses of the lateral ventricles."

Of the Urodeles, Burekhardt ('91) has described extensive plexuses developing both from the tela chorioidea telencephali medii and from the tela chorioidea diencephali in *Ichthyophis*. Warren ('05) shows the enormous diencephalic plexus of *Necturus* absorbing also the entire caudal wall of the velum transversum. There is a plexus in the roof of the fourth ventricle. The lateral telencephalic plexuses are present and arise from the base of the median telencephalic plexus (plexus inferioris) as has been attested by Mrs. Gage ('93), Studnicka ('93), Warren ('05) and Burekhardt ('91). Burekhardt ('91) says of *Ichthyophis*: "Die Plexus der Hirnhemisphären aber spalten sich je in zwei Stämme von denen der eine sich gegen das Zwischenhirn ausstreckt, und in der Folge zuerst sich in Zweige spaltet, indess der andere in den Hemisphärenventrikel eindringt und sich sodann in zwei Zweige spaltet, einen nach rückwärts umbiegenden, welcher den Ventrikeltheil des Temporallappens und einen, welcher das übrige Vorderhirn versorgt." And Warren ('05) writes concerning *Necturus*: "The telencephalic plexus develops from the paraphysal arch." "The plexuses of the hemispheres arise on either side from the origin of the telencephalic plexus and pass into the lateral ventricles."

Concerning the Chelonia, Humphrey ('94) shows a plexus arising from the tela chorioidea telencephali medii in *Chelydra serpentina*, as does also C. L. Herrick ('91) in *Cistudo*. Warren ('11) states that the plexus is not present in *Chrysemys marginata* and with this statement my observations agree. The "two

paired masses growing backward from the origin of the lateral plexus into the diencephalon" of which Warren writes do not seem to me to be at all homologous with the median telencephalic plexus. They arise too far posterior on the plexus; are at no time connected with the roof plate; and are merely prolongations of the lateral plexus. The diencephalic plexus is not so well developed as in *Urodeles* but is present in all forms, as is also the plexus of the fourth ventricle. Humphrey ('94) shows in *Chelydra serpentina* the lateral telencephalic plexus arising from the base of the median telencephalic plexus and passing into the lateral ventricle. According to Warren ('11), in *Chrysemys marginata*, "The plexus chorioideus lateralis springs from the paraphysal arch immediately in front and lateral to the mouth of the paraphysis, figure 25, and invaginates the dorso-mesial wall of the hemisphere."

In considering the Mammalia, considerable space will be given to interpreting accurately the velum and paraphysis. This is rendered necessary if one is profitably to homologize the lateral telencephalic plexus of Mammalia with the same structure in the lower vertebrates. Since, as we shall see later, the paraphysis never appears in Mammalia except as an arch of the roof plate of the telencephalon in early stages of development, it will be referred to as the paraphysal arch. Minot ('01) used the term to apply to the entire roof plate of the telencephalon between the velum transversum and the lamina terminalis, a sense in which it is no longer used.

The only observations of value dealing with the Monotremes are those of Th. Ziehen ('05) on *Echidna hystrix* and G. Elliot Smith ('97) on *Ornithorhynchus*, and these leave much to be desired. Smith describes the origin of the lateral telencephalic plexuses and a structure which he says "constitutes the paraphysis of Selenka." I do not know what evidence he had for stating that the structure he describes is the paraphysis of Selenka for so far as I have been able to determine, Selenka has nowhere a description of the paraphysis but merely the bald statement that it is present in Marsupials. Smith's statement probably implied no more than that he interpreted this structure as the

paraphysis. Extracts from this paper follow and will be discussed later.

The lamina supraneuroporica takes a sudden bend backwards (fig. 3) to form a horizontal band, which gives origin in many lowly vertebrates to the plexus inferioris, and in higher animals to the plexus lateralis as well, or exclusively. In the specimen under consideration, however, although the plexus laterales do not actually spring from this lamina, they are formed from the caudal prolongation of its lateral parts. . . . In describing the structures met with in a medial sagittal section it was mentioned that the dorsal part of the (actual) anterior wall of the median cavity of the forebrain was bulged out to form a large sac. The corresponding structure is well seen in the early embryo of *Parameles*. . . . In the *Platypus* embryo, however (fig. 2), a well developed choroidal fold extends from the superior commissure to the lamina from which the lateral plexus arises, completely invaginating the paraphysis (figs. 7, 9 and 15) in the middle line. In *Platypus* the transition from optic thalamus to paraphysis is a very gradual one, so that in examining a series of coronal sections the lateral walls of the diverticulum would seem to be merely the forward continuation of the ependymal layer of the Flügelplatten (fig. 15).

As a matter of fact, the appearances in this series of coronal sections are not deceptive at all, the sac being undoubtedly just what it appears to be, an anterior pouch of the choroid plexus of the diencephalon, the lateral walls of the pouch being actually the forward continuation of the Flügelplatten. A glance at figures 1, 2 and 3 will make this quite apparent. The similarity would be much more obvious if figure 3 were from a coronal instead of a transverse section. In front of this pouch lies the velum transversum and then the roof plate of the telencephalon (lamina supraneuroporica as he calls it), from the caudal prolongations of the lateral parts of which arise the lateral telencephalic plexuses. In a later article, Smith ('03) reaffirms his belief that the anterior extremity of the lateral telencephalic plexus arises from the roof plate. The embryo is doubtless too far advanced to show the true paraphysal arch.

Ziehen's work on *Echidna* is eminently unsatisfactory from the standpoint of this discussion, because the sections he shows invariably skip the anterior end of the lateral telencephalic plexus. He has but one suggestive statement referring to this region:

Im Bereich des Sulcus hemisphaericus ist die mediale Hemisphärenwand verdünnt und taschewartig in das Hemisphärenlumen eingestülpt. Diese Tasche entspricht dem Plexus chorioideus ventriculi lateralis. Sie öffnet sich also in den Sulcus hemisphaericus (und zwar in seine laterale Wand) in der Decke des Foramen Monroi und communicirt sowohl mit der Siehelspalte wie mit dem hinten absteigenden zwischen Zwischenhirn und Hemisphärenhirn gelegenen Abschnitt des Sulcus hemisphaericus.

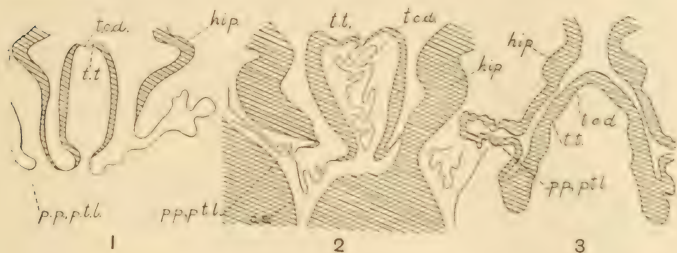


Fig. 1 Coronal section from the forebrain of an embryo of *Parames nasuta*. Copied from Smith. Labels mine.

Fig. 2 Coronal section from the forebrain of a foetal *Ornithorhynchus*. Copied from Smith. Labels mine.

Fig. 3 Transverse section through the forebrain of a 19 mm. human embryo. H 173, Chicago Embryological Collection. Slide 21, Section 11. $\times 13\frac{1}{2}$.

REFERENCE LETTERS

- | | |
|--|--|
| <i>a.a.c.t.l.</i> , anterior area chorioidea telencephali lateralis | <i>p.a.</i> , <i>p.t.l.</i> , pars anterior plexus telencephali lateralis |
| <i>c.o.</i> , chiasma opticum | <i>par.</i> , paraphysis |
| <i>c.p.</i> , commissura posterior | <i>p.p.</i> , <i>p.t.l.</i> , pars posterior plexus telencephali lateralis |
| <i>c.s.</i> , corpus striatum | <i>r.inf.</i> , recessus infundibuli |
| <i>d-t.gr.</i> , di-telencephalic groove | <i>r.m.</i> , recessus mamillaris |
| <i>ep.</i> , epiphysis | <i>r.post.</i> , recessus postopticus |
| <i>f.c.</i> , fissura chorioidea | <i>r.pre.</i> , recessus preopticus |
| <i>f.M.</i> , foramen interventriculare | <i>t.c.d.</i> , tela chorioidea diencephali |
| <i>hem.</i> , hemisphere wall | <i>t.c.t.m.</i> , tela chorioidea telencephali medii |
| <i>hy.</i> , hypothalamus | <i>t.f.</i> , taenia fornicis |
| <i>l.t.</i> , lamina terminalis | <i>th.</i> , thalamus |
| <i>mes.</i> , mesencephalon | <i>t.r-p.</i> , telencephalic roof-plate |
| <i>n.a.t.</i> , nucleus anterior thalami | <i>t.t.</i> , taenia thalami |
| <i>n.o.</i> , nervus opticus | <i>v.t.</i> , velum transversum |
| <i>p.a.c.t.l.</i> , posterior area chorioidea telencephali lateralis | |

The 'Sichelspalte' I understand to be the cleft between the hemispheres in front of the diencephalic roof. The lateral telencephalic plexus, then, must extend anteriorly beyond the velum transversum into the roof plate of the telencephalon.

Ziehen also is inclined to interpret the anterior extremity of the diencephalic roof in *Echidna* as the paraphysis. He remarks:

Das ganze Bild erinnert an die Paraphyse mancher Reptilien. Ich stehe auch nicht an, diese leichte forcere Zuspitzung des hinteren Kuppelgebietes der Paraphyse homolog zu sitzen, wie im vergleichenden Abschnitt specieller erörtert werden soll. Eine tiefere Einsenkung hinter der Zuspitzung—etwa im Sinne eines Velum transversum—fehlt. Vor der Zuspitzung beginnt sofort die Einsenkung der Fossa interhemisphaerica.

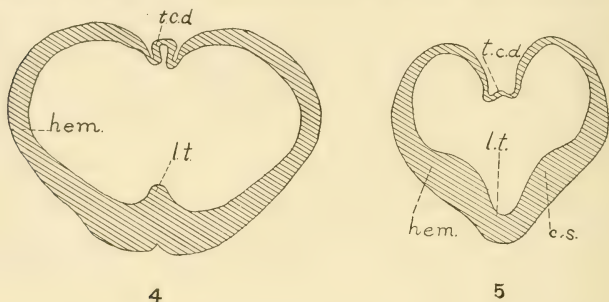


Fig. 4 Section from the forebrain of a $6\frac{1}{2}$ mm. embryo of *Echidna hystrix*. $\times 30$. Copied from Ziehen. Labels mine.

Fig. 5 Transverse section from the forebrain of a 14 mm. pig. no. 18, Chicago Embryological Collection. Slide 5, section 19. $\times 25$.

In an older embryo of which he says, "Sehr bemerkenswerth is wiederum das Verhalten des Kuppelgebietes des Zwischenhirns gegen die Fossa praediencephalica hin," he describes nothing resembling a velum transversum and a comparison of figures 4 and 5 will show the resemblance of his 'Kuppelgebietes' to the anterior pouch of the tela chorioidea diencephali of a pig embryo. If the plane of section in figure 5 were homologous with that of figure 4 the resemblance would be much more striking. If it be really the paraphysis, it forms a good transition stage to the condition found in higher Mammalia.

Observations on the Marsupial brain are remarkably meagre. I have been able to find but two statements bearing on the subject. Broom ('97) says of a 14.8 mm. embryo of *Trichosurus vulpecula*: "The choroid folds into the lateral ventricles, is partly formed, and the paraphysis well marked." He has no figures of sections. Selenka ('91) has a simple statement that the paraphysis is present in Marsupials, presumably in the opossum. "Bis jetzt habe ich die Paraphyse bei embryonen von Haifischen, Reptilien, und Beuteltieren beobachtet, zweifle jedoch nicht, dass sie allen Wirbeltieren gemeinsam ist." That is all, but I hope soon to fill this gap.



Fig. 6 Sagittal section of the forebrain of a 15 mm. pig. Copied from Johnston. $\times 10$. Labels mine.

Fig. 7 Sagittal section of the forebrain of a 11 mm. embryo of *Erinaceus europaeus*. Copied from Grönberg. $\times 10$. Labels mine.

Thanks mainly to Grönberg, the brain of the Insectivora is better known. His work was done with *Erinaceus europaeus*. Ziehen ('06) says of *Tupaja*, "Die Verhältnisse gleichen den für den Igel beschriebenen in hohem Masse."

Grönberg's figures 25, 26, and 27 show clearly the velum transversum and paraphysal arch although he is loath to call them so. A comparison of figures 6 and 7 will make this plain. He describes also plexuses in the diencephalon and fourth ventricle. Of the lateral telencephalic plexus he writes:

Die Adergeflechtsfurche entsteht am frühesten. Sie ist schon bei meinem Stadium C (11 mm.) vorhanden und sowohl ausgebildet, dass ihre erste Entstehung sicher auf einem bedeutend jüngern Stadium zu suchen ist. Doch findet sich auf Stadium B (8 mm.) noch keine Spur einer Faltenbildung. Die Form und das Aussehen der Falte ergeben sich aus den Figg. 52 und 53. Man sieht, dass sie in ihrem vordern Theil weiter in die Hemisphärenhöhle hineinreicht, als es mehr caudalwärts der Fall ist. Es zeigt sich auch, wenn man eine Schnittserie durchmustert, dass die Falte nach hinten allmählich kleiner wird und schliesslich nur eine leichte Einbuchtung darstellt, welche nach hinten ganz allmählich verstreicht. Der vordere Theil dagegen verhält sich ganz anders. Die Falte ist hier tief und erstreckt sich mit ihrem freien Rand weiter nach vorn als die mit der übrigen Hirnwand in Verbindung stehende basis. . . . Auf den folgenden Stadien vergrössert sich die Adergeflechtsfalte bedeutend, besonders in ihr vorderer, freier Theil,

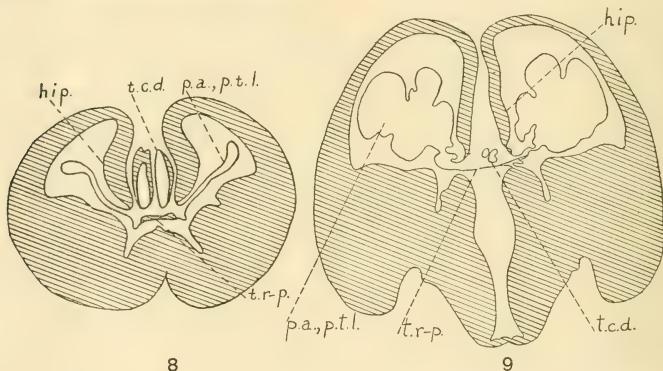


Fig. 8 Cross section through the forebrain of a 15 mm. embryo of *Erinaceus europaeus*. Copied from Grönberg. $\times 10$. Labels mine.

Fig. 9 Cross section through the forebrain of a 32 mm. human embryo. H41, Chicago Embryological Collection. $\times 25$. Slide 29, Section 6.

One would conclude from this description that the anterior end of the lateral plexus had arisen the earlier, and that it arises from the roof plate of the telencephalon is clearly shown in his figure 54, which is here reproduced with a section from a human embryo for comparison (figs. 8 and 9). Again if the section of the human embryo were a coronal instead of a transverse section, the similarity would be more obvious.

Of the forms between Insectivora and Man nothing of value is known. Ziehen ('06) shows sections of the lateral telencephalic plexus of *Tarsius spectrum*, a prosimian, but not in the proper plane to be of value, and again describes the anterior pouch of the diencephalic roof as the paraphysis.

Sehr beachtenswert ist auch, dass unmittelbar hinter der Fossa praediencephalica die epitheliale Decke des Zwischenhirns sich zu einer stulzen Falte, welche an die Paraphyse erinnert, erhebt und dass erst einige Schnitte weiter occipitalwärts diese steile Falte durch den mediane Plexus des 3. Ventrikels eingestülpt wird.

In an earlier paper, the author (Bailey, '15) called attention to the true homologue of the paraphysis in the human embryo and insisted upon its position in the telencephalic roof plate just anterior to the velum transversum. Previously, Streeter ('12), Francotte ('94) and D'Erechia ('96) had written of the paraphysis in the human embryo. Streeter homologizes the anterior pouch of the diencephalic roof with the paraphysis, and from a comparison of Francotte's figures with sections of embryos of approximately the same stage, I am convinced that he mistook the same structure for the paraphysis. D'Erechia shows a section from a 30 mm. human embryo in which he labels a structure 'paraphysis' which seems to me to be merely an oblique section of the lateral telencephalic plexus.

In his model of a 13.6 mm. human embryo, His shows clearly the lateral telencephalic plexus coming off lateral to the paraphysal arch, but his statement ('04) of the origin takes no account of this fact. "Sein dem Thalamus angehefteter Randstreifen bleibt endymal und in ihm bildet sich die Fissura chorioidea, von der aus die Epithelfaltungen des Corpus chorioideum in den Seitenventrikel sich einstülpen."

Höchstetter ('13) in his account of the development of the lateral telencephalic plexus in the human embryo shows one figure (fig. 6) of a section through the plexus which passes also through the telencephalic roof plate, but his description contains nothing concerning the origin of this part of the plexus.

D'Erechia ('96) considers the lateral telencephalic plexus to be derived from the velum transversum: "Per questa parte volga

tutto quello che si e detto per la cavia. Dal velum per diverticoli laterali di formano i plessi emisferici inferiori destro e sinistro, dei quali uno e disegnato nella fig. 28."

Finally the author (Bailey, '15) has presented an account of the development of the lateral telencephalic plexus which it is the purpose of the present paper to supplement.

Concerning these structures in other groups of vertebrates this discussion is not so much concerned, but a few words at least about the lateral plexus in other vertebrates will not be out of place. This plexus is not present in Teleosts (Burekhardt, '94) nor Anura (Herrick, '10). In Lacertilia its development has been described by Tandler and Kantor ('07) and Warren ('11) and agrees in all essential respects with that hereinafter detailed for *Chrysemys marginata*. In examining some young alligators in the collection of Dr. Elizabeth Crosby, the posterior part of the lateral telencephalic plexus was found to be present, but very small and poorly developed. The brain of an adult pigeon was also examined and the lateral telencephalic plexus was seen arising from a plexus formation in the roof of the telencephalon, but of the posterior part of the plexus there was no sign except a plexus of blood vessels along a thin but uninvginated medial hemisphere wall. Dr. C. Judson Herrick suggested that this may represent a stage in the phylogenetic development of the plexus. Mrs. Gage ('95) figures the brain of the embryo of the English sparrow. Both the figures and the context show that the lateral telencephalic plexuses arise from the median telencephalic plexus and just lateral to the paraphysis, apparently not invaginating the medial hemisphere wall. She writes: "In the young sparrow (g. 2) the paraphysis occurs in the midst of a mass which gives off the paraplexuses, and it opens directly dorsad of the portas, i.e., into the aula. . . . It is noticeable in the older embryo that the union of the auliplexus with the paraplexuses lies dorsad of the porta (fig. 14)." The works of Neumayer ('99) and Hochstetter ('98) contain nothing of value for the present discussion. The two invaginations which they describe are temporary and unimportant. A similar condition is shown in my figure 19 (Bailey, '15). D'Erchia ('96)

considers the lateral plexus in the guinea pig to be derived from the velum transversum. "E dal velum che si origina il plesso del III ventricolo e i plessi coroidi emisferici destro e sinistro." It is interesting also to note that although Tilney ('15) correctly labels the paraphysal arch in models of the brains of young cat embryos, in later stages he invariably transfers the label to the anterior pouch of the diencephalic roof. Johnston ('09) has correctly interpreted the paraphysis in pig embryos, but his statement that the lateral telencephalic plexus appears as a folding of the anterior wall of the velum transversum is misleading for he immediately follows with the statement that it is separated from the velum by the paraphysal arch.

In the angle between the vesicle and the diencephalon appears the chorioid plexus pushing into the lateral ventricle. It appears as a folding of the anterior wall or limb of the velum transversum and its lateral prolongation. In this way appears the chorioid fissure whose further history need not be traced. Near the median line the plexus appears as a fold projecting into the interventricular foramen and separated from the velum transversum by the paraphysal arch.

MATERIAL AND METHODS

The material on which this study is based consists of a series of twelve embryos of *Chrysemys marginata* from my own collection, ranging in size from 5.1 mm. greatest length to one having a carapace 10.6 mm. long, and an 8.8 mm. embryo from the Harvard Embryological Collection, very kindly loaned to me by the Harvard Laboratory. All of my material was fixed in Zenker; stained in bulk with borax carmine; embedded by the celloidin-paraffin method; cut at 10 μ in transverse series; and counterstained on the slide with orange G. The excellent preservation of the form relations of the delicate membranous portions of the brain may be attributed to the method of embedding. The embryos were all passed from 95 per cent alcohol to ether-alcohol; then through 0.5, 1 and 2 per cent celloidin; hardened in chloroform alcohol, and cleared in benzol, before embedding in paraffin. The Harvard embryo is cut in 10 μ sagittal sections and stained with borax carmine and eosin.

The forebrains of four embryos—1 a, greatest length 5.1 mm.; H. E. C. no. 1433, greatest length 8.8 mm.; 5 b, carapace 8.6 mm.; and 4 b, carapace 10.6 mm.—were reconstructed by the Born method at a magnification of 100 diameters. Millimeter plates were used and every section drawn. It was necessary to dissect the models rather extensively to expose the fissura chorioidea. The models were stacked from a side view of the head drawn from a photograph, with the exception of the Harvard embryo. This latter embryo was cut sagittally and the stacking was guided by the epiphysis and paraphysis. Since the embryo was not cut in an exactly sagittal plane, after the paraphysis and epiphysis had passed out of the plane of section, most of the lateral telencephalic plexus had been stacked and the remaining sections were added with no other guide except comparison with other models.

DESCRIPTION

This description will be confined to the lateral telencephalic plexus, since concerning the other plexuses I find no reason to differ from Warren's account, with the exception noted in the history.

The main landmarks of the region in which the lateral telencephalic plexus develops are already laid down in an embryo of 5.1 mm. greatest length. Figure 26 shows the roof plate of the forebrain in such an embryo and figure 10 shows the same region schematically represented. The roof plate of the telencephalon, back of the lamina terminalis, appears as a triangular area (only half of it shown in figure 26) its base formed by the velum transversum and its apex lying at the posterior end of the lamina terminalis while from its center arises the paraphysis. The lateral sides of the triangle are formed by the taeniae fornices. At the lateral angles of the triangle, taenia fornices, velum transversum, taenia thalami and di-telencephalic groove meet. It is not possible accurately to determine this point in the embryo under consideration because the roof-plate has been but imperfectly differentiated histologically, but in later stages these angles of the triangle may be easily located.

The earliest unquestionable appearance of the lateral telencephalic plexus is in the Harvard embryo, 8.8 mm. in length. It appears here as a crescent-shaped ridge projecting into the lateral ventricle, with two more strongly developed points showing as small elevations (fig. 22). The anterior extremity of the plexus lies clearly in the roof of the telencephalon lateral to the paraphysis and medial to the taenia fornicia. The taenia fornicis appears in figure 22 as a ridge apparently in the medial hemisphere wall. It bears here a very close superficial resemblance to the hippocampal ridge in a young human embryo, but its later development and internal structure show plainly that

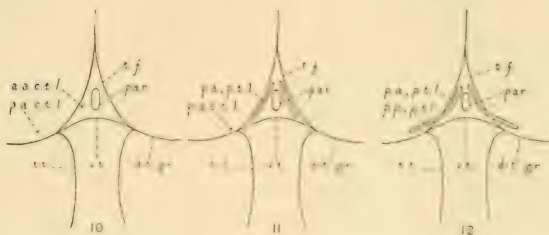


Fig. 10 Diagram of the region around the paraphysis in the roof of the fore-brain of a 5.1 mm. embryo of *Chrysemys marginata*.

Fig. 11 Diagram of the same region in an embryo of 8.8 mm.

Fig. 12 Diagram of the same region in an embryo having a carapace of 8.6 mm.

it is the taenia fornicis. Its apparent position in the medial hemisphere wall is an illusion produced by the invagination of the plexus between it and the paraphysis. The triangular area of the telencephalic roof plate is well marked and the lateral angles can be determined. A sagittal section through the triangle is practically a straight line, except for the evagination of the paraphysis (figs. 13 and 21) as are also parasagittal sections (fig. 14). Nevertheless the lateral angles are depressed and the sides of the triangle are concave outward. Transverse sections through this region are therefore curved and convex upward (fig. 21). The velum transversum sharply delimits the telencephalic roof plate at the base of the triangle (fig. 14).

Figure 15 shows a parasagittal section still farther laterally through the triangle. The velum transversum is obvious, the plexus invaginating the roof plate medial to the taenia fornicis and tilting the portion of the roof between itself and the taenia. Above and lateral to the taenia fornicis the brain wall is histologically differentiated; medial it is ependymal. This difference is much more apparent in later stages (fig. 17). Posteriorly the plexus is less well developed and as we approach the lateral angles of the triangle, plexus, velum, and taenia fornicis tend to merge into one groove which becomes continuous with

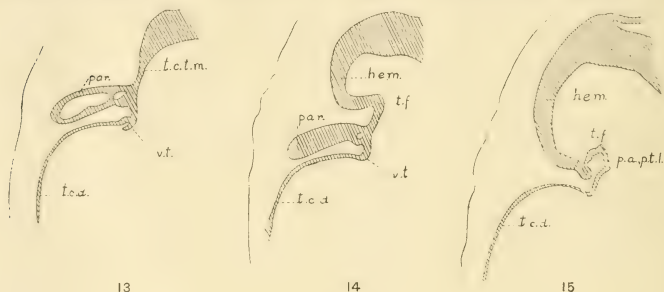


Fig. 13 Sagittal section no. 135 from an 8.8 mm. embryo of *Chrysemys marginata*. Harvard Embryological Collection, no. 1433. $\times 33\frac{1}{2}$.

Fig. 14 Parasagittal section no. 130 from the same embryo.

Fig. 15 Parasagittal section no. 124 from the same embryo.

the di-telencephalic groove. Lateral and anterior to the point where the taenia thalami meets the di-telencephalic groove and velum transversum the hemisphere wall is massive and uninvaginated. It would seem, then, that in this embryo, the lateral telencephalic plexus lies entirely in the roof plate of the telencephalon. The condition is diagrammatically represented in figure 11.

Figures 23 and 24 depict the region around the foramen of Monro in an embryo with a carapace 8.6 mm. long, about 14 mm. greatest length. Figure 23 looks at the foramen of the right half of the brain from the medial side. The cephalic end of the

model is, consequently to the left. The paraphysis which had been removed is represented by a line of white dashes. Figure 24 looks at the same foramen from the lateral side. The model is therefore reversed and the cephalic end points to the right.

The condition of the roof-plate is diagrammatically represented in figure 12. The differentiation between hemisphere wall and

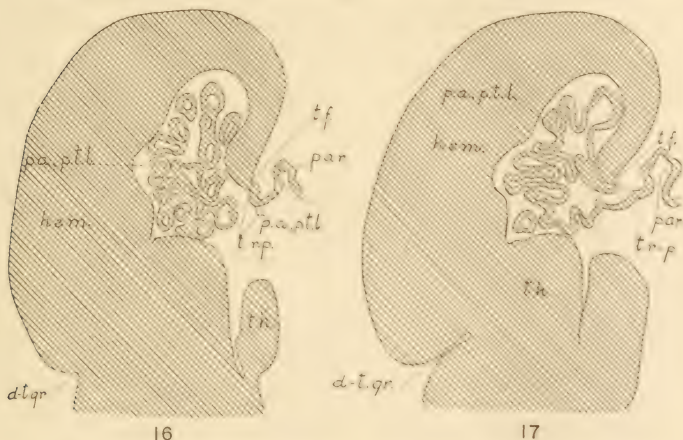


Fig. 16 Transverse section from the forebrain of an embryo of *Chrysemys marginata* having a carapace 5.6 mm. in length. Embryo 5 b, slide 13, sect. 11. $\times 33\frac{1}{2}$.

Fig. 17 Transverse section of the same embryo; section 16, slide 13. $\times 33\frac{1}{2}$.

roof plate is clear and the taenia fornicis obvious (figs. 16 and 17). The anterior extremity of the lateral telencephalic plexus arises plainly from the telencephalic roof plate lateral to the paraphysis and medial to the taenia fornicis (fig. 16). Figure 17 shows a section posterior to figure 16 through the main body of the plexus, the plexus still lying in the roof plate. Figure 18 is of a section still farther posteriorly. The plexus is here shown crossing the taenia fornicis into the medial hemisphere wall. The taenia fornicis is now medial to the plexus and drop-

ping down to meet the anterior nucleus of the thalamus, which it does in a few sections and becomes continuous with the taenia thalami. In figure 19, much farther posteriorly, the taenia thalami is present, the plexus being entirely in the medial hemisphere wall. The portion of the plexus arising from the medial hemisphere wall is very poorly developed (fig. 19) but its area of invagination is extensive (fig. 24).

In later stages this posterior part of the plexus develops more rapidly and overshadows the other. Figure 25 shows a lateral

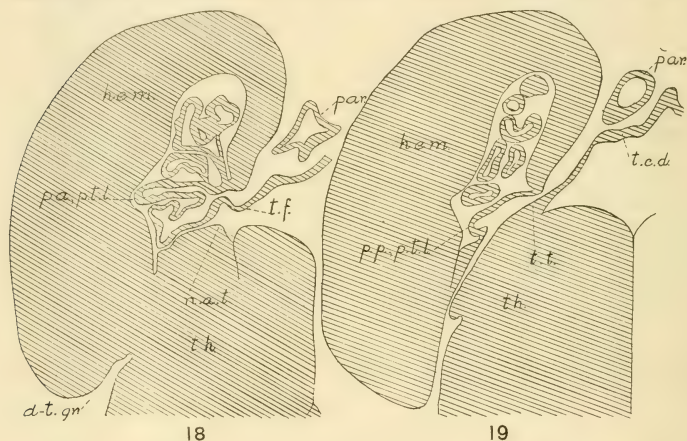


Fig. 18 Transverse section of the same embryo as figure 16. Slide 13, section 22. $\times 33\frac{1}{2}$.

Fig. 19 Transverse section of the same embryo; section 5, slide 14. $\times 33\frac{1}{2}$.

view of the region around the foramen of Monro in an embryo with a carapace of 10.6 mm. A pen sketch of the entire model is appended (fig. 27) showing the region represented in figure 25. (Figure 24 is of a homologous region in a younger embryo.) The fissura chorioidea appears merely as a big hole in the medial hemisphere wall; all the landmarks are lost. In still later stages, this hole becomes reduced to a long narrow slit.

The development of the plexus in size and shape is so well discussed and figured by Warren ('11) that it will not be considered here.

DISCUSSION

From the foregoing history and description, and from an analysis of the remaining literature, for in the history are included only the most important papers and especially those dealing with the lateral telencephalic plexuses, it will be seen that there are certain definite regions of the brain wall wherein choroid plexuses develop. These regions and the plexuses which develop from them may be tabulated as follows:

- Tela chorioidea telencephali medii—Plexus telencephali medius
- Anterior area chorioidea lateralis telencephali—Plexus telencephali lateralis (below Chelonia)
 - { Anterior area chorioidea lateralis telencephali—Plexus telencephali lateralis (Chelonia and above)
 - { Posterior area chorioidea lateralis telencephali (hemisphericici)—Plexus telencephali lateralis (Chelonia and above)
- Velum transversum—Plexus velares
- Tela chorioidea diencephali—Plexus diencephali
- Tela chorioidea mesencephali—Plexus mesencephali
- Tela chorioidea myelencephali—Plexus myelencephali

Tilney ('15) has suggested that the saccus vasculosus should be reckoned with the choroid plexuses in the forms where it is present and in this opinion I concur. It is best developed in those forms in which the diencephalic plexus is rudimentary or absent, i.e., in Cyclostomes, Selachians and Ganoids. The plexus formation is very poorly developed in Urodeles and never again present. There should be added to the above therefore:

Recessus infundibularis (posterior wall)—Saccus vasculosus.

The myelencephalic plexus arises in the roof of the fourth ventricle, tela chorioidea myelencephali, in every known vertebrate above Amphioxus.

The mesencephalic plexus is found only in Petromyzon, where it arises from the mesencephalic roof, tela chorioidea mesencephali.

The diencephalic plexus arises from the tela chorioidea diencephali. Appearing first in Cyclostomes, but very poorly developed, it disappears almost entirely in Selachians, where the tela chorioidea diencephali is almost completely absorbed

by the overgrowth of the velum transversum. In Ganoids, the tela chorioidea diencephali begins to emerge, forming a thin walled sac, and in Urodeles is again invaginated by an enormous diencephalic plexus. It may be that the diencephalic plexus in Selachians and Ganoids is represented by the choroidal folds on the posterior limb of the velum, developing from the anterior portion of the tela chorioidea diencephali which has been drawn down into the velum transversum by the overgrowth of the latter. The diencephalic plexus is present in all forms above Urodeles but is never again so well developed.

The velar plexuses are present only in Selachians, Ganoids, and Urodeles. They may involve either the diencephalic limb or telencephalic limb, or the entire velum transversum. The choroidal folds on the diencephalic limb have been homologized, in Selachians and Ganoids, with the diencephalic plexus of other forms, and with some reason. The choroidal folds on the anterior limb are not homologous with either the median or lateral telencephalic plexuses.

The median telencephalic plexus arises from the tela chorioidea telencephali medii, just in front of the paraphysis (paraphysal arch of Mammalia), from the Selachians to the Chelonia, inclusive, with the apparent exception of the Ganoids. It is not constantly present in Chelonia and is never found in Mammalia. Its development seems to be in inverse ratio to the degree of development of the lateral telencephalic plexus and the latter in direct ratio to the size of the hemispheres.

The lateral telencephalic plexus is found in all groups of vertebrates from the lowest to the highest in the line of ascent of Mammals with the exception of the very lowest, *Amphioxus* and the *Cyclostomes*. Apparently sporadically and imperfectly developed in Selachians and Ganoids, it is present constantly thereafter. We have stated previously that it is absent in *Teleosts* and *Anura*, with which we are not concerned. In all forms below Chelonia, it develops in what I have called elsewhere (Bailey, '15) the anterior lateral telencephalic chorioidal area, in the roof plate of the telencephalon between the paraphysis and the taenia fornicis of the medial hemisphere wall. Where the median

telencephalic plexus is strongly developed the lateral plexus appears to be an appendage of it (Necturus, Warren); where the velum is involved in an extensive plexus formation, the lateral plexus appears to be an appendage of it (Acanthias, Minot); but always arises from the region of the telencephalic roof plate described above, and medial to the taenia fornicis.

With the Chelonia comes a change. The lateral plexus arises in the anterior area chorioidea lateralis telencephali as has been previously described but in its later development crosses the taenia fornicis and invaginates also the posterior area chorioidea lateralis telencephali in the medial hemisphere wall. Such a condition is found also in the Gecko brain (Tandler and Kantor). This involvement of the medial hemisphere wall comes more and more to predominate in the development of the lateral plexus as the hemispheres come more and more to dominate the development of the telencephalon, but even in the highest Mammalia the anterior extremity of the lateral choroid plexus develops from the roof plate of the telencephalon and the evidence may briefly be summarized here:

(a) Elliot Smith ('97) in describing the brain of a foetal Ornithorhynchus described the lateral choroidal plexus as arising from the continuation backwards of the horizontal part of the lamina supranucuporica, which term he used to include all the roof plate of the telencephalon between the velum transversum and the lamina terminalis. Again in 1903, he reiterated his belief that the anterior extremity of the lateral choroidal plexus arises from the roof plate.

(b) Th. Ziehen's figures of Echidna do not show the anterior extremity of the lateral plexus, but he states that it opens into the Siehelspalte, that is, into the cleft between the hemispheres anterior to the diencephalon, as well as the sulcus hemisphaericus (di-telencephalic groove).

(c) Observations on the Marsupials up to present reveal nothing.

(d) Grönberg states of Erinaceus europaeus that the anterior extremity of the lateral plexus is better developed in his earliest stage than the posterior extremity and apparently arises first.

This holds true of all mammals and is what one would expect from the phylogenetic history of the plexus. His figure 42 of a section anterior to the velum shows clearly the anterior extremity arising from the roof plate of the telencephalon.

(e) The earliest appearance of the plexus in the human embryo occurs in His' embryo of 13.6 mm. and his model shows clearly the plexus arising just lateral to the paraphysal arch.

(f) Hochstetter ('13) figures a section of a human embryo showing the anterior extremity of the plexus, and the author recently (Bailey, '15) has described three human embryos showing the plexus extending into the roof plate alongside the paraphysal arch anterior to the velum transversum.

(g) Collateral evidence is derived from the development of the lateral plexus in other mammals, for example, Johnston ('09) states that in the pig the plexus arises from the anterior limb of the di-telencephalic groove but is separated medially from the velum transversum by the paraphysal arch.

CONCLUSIONS

1. The lateral telencephalic plexus of *Chrysemys marginata* arises from the anterior area chorioidea lateralis telencephali in the roof plate of the telencephalon between the paraphysis and the taenia fornicis of the medial hemisphere wall and in its later development oversteps the taenia fornicis just anterior to the point where taenia fornicis, taenia thalami, velum transversum and di-telencephalic groove meet, and invaginates also the posterior area chorioidea lateralis telencephali in the medial hemisphere wall, just anterior to the di-telencephalic groove. The pars anterior plexus telencephali lateralis which develops from the anterior area chorioidea lateralis telencephali in early stages is much larger and better developed than the pars posterior plexus telencephali lateralis, but in later stages develops much less rapidly than the pars posterior.

2. All the data at present available support the conclusion that the lateral telencephalic plexus arises from the anterior area chorioidea lateralis telencephali in the roof plate of the telencephalon between the paraphysis (or paraphysal arch) and the

taenia fornicis in all vertebrates where it is present, but that in Chelonia and the forms above, it oversteps the taenia fornicis and invaginates the posterior area chorioidea lateralis telencephali in the medial hemisphere wall and that this latter portion comes more and more to dominate its development as we ascend the vertebrate scale.

I wish here to express my gratitude to Dr. S. Walter Ranson for his unvarying kindness and interest throughout the progress of this work.

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PLATE 1

EXPLANATION OF FIGURES

The reference letters for all figures are found on page 513. It is impossible to portray all the topography of the region around the foramen interventriculare by means of drawings. For aid in orientation, the position of the principal landmarks is indicated on the models as follows: the taenia fornicis by a line of dashes; the velum transversum by a line of crosses; the taenia thalami by dots; and the di-telencephalic groove by dots and dashes alternating. The cross sections will also aid. On figures 22 and 23 the positions of the sections are indicated by arrows. Since the models are tilted, the arrows indicate accurately only the points where the sections cross the lateral telencephalic plexus.

20 Median view of the forebrain of a 5.1 mm. embryo of *Chrysemys marginata*. Embryo 1 a. $\times 50$.

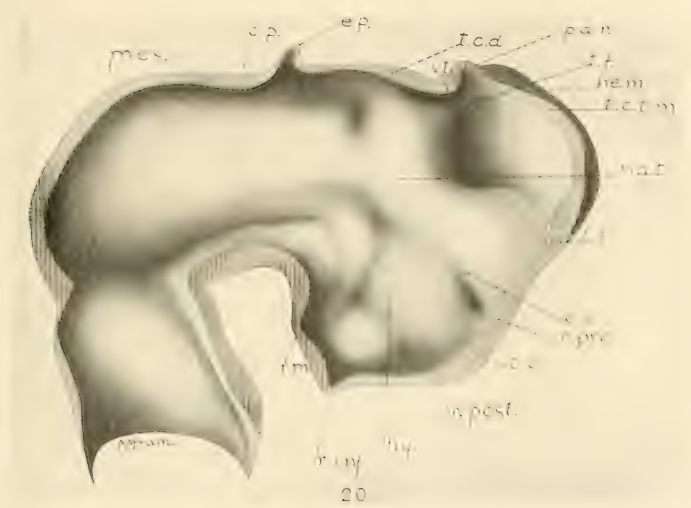
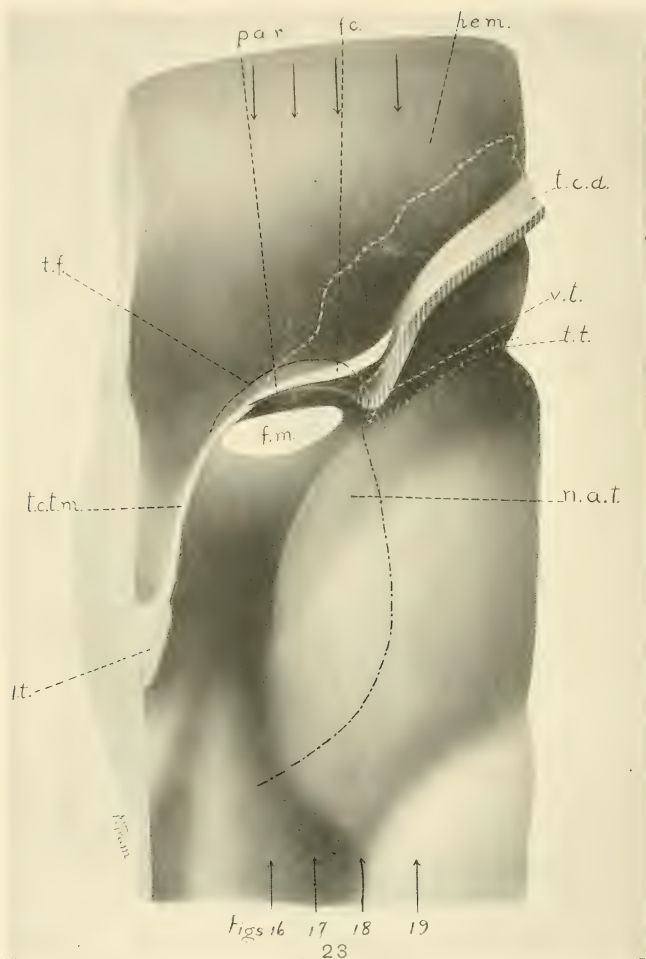


PLATE 2

EXPLANATION OF FIGURES

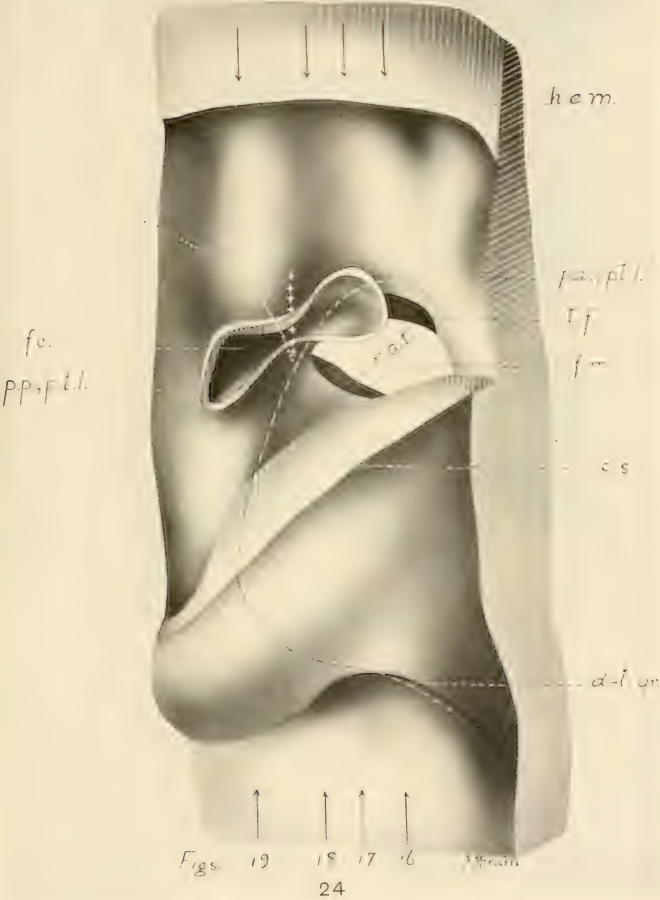
- 21 Median view of the forebrain of an 8.8 mm. embryo of *Chrysemys marginata*. Embryo 1433. H.E.C. $\times 40$.
- 22 Lateral view of the forebrain of an 8.8 mm. embryo of *Chrysemys marginata*. Embryo 1433. H.E.C. $\times 40$. Lateral hemisphere wall removed.





EXPLANATION OF FIGURES

23 Median view of the region around the foramen interventriculare in an embryo of *Chrysemys marginata* having a carapace 8.6 mm. in length. Embryo 5 b. $\times 100$. In order to show the fissura chorioidea it was necessary to remove the paraphysis and represent the fissure slightly higher than it really is.



EXPLANATION OF FIGURES

24 Lateral view of the same region as in figure 23. Lateral wall of the hemisphere and plexus telencephali lateralis removed, exposing the fissura chorioidea. Embryo 5 b. $\times 100$.

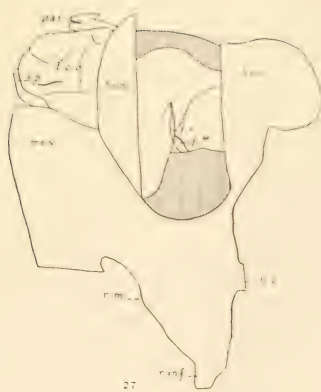
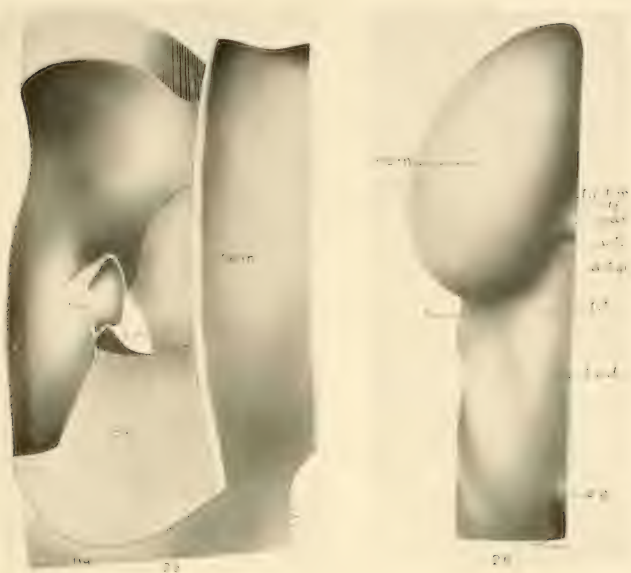
PLATE 5

EXPLANATION OF FIGURES

25 Lateral view of the region around the foramen interventriculare in an embryo of *Chrysemys marginata* having a carapace 10.6 mm. in length. Embryo 4 b. $\times 66\frac{2}{3}$. Lateral hemisphere wall and lateral telencephalic plexus removed, exposing the fissura chorioidea.

26 Dorsal view of the forebrain of a 5.1 mm. embryo of *Chrysemys marginata*. Embryo 1 a. $\times 80$.

27 Pen sketch of model of entire forebrain of embryo 4 b, showing the area from which figure 25 was taken. $\times 25$.



THE STRUCTURE OF THE THIRD, FOURTH, FIFTH, SIXTH, NINTH, ELEVENTH AND TWELFTH CRANIAL NERVES

SUMNER L. KOCH

From the Anatomical Laboratory of the Northwestern University Medical School

FIVE FIGURES

Following the demonstration of unmyelinated fibers in the spinal nerves and in the vagus nerve by means of the pyridine-silver technique (Ranson, '11 and '12; Chase and Ranson '14), Professor Ranson suggested the application of the same method to the study of certain of the cranial nerves, with especial reference to the presence or absence of unmyelinated fibers.

The nerves studied were the oculomotor, trochlear, trigeminal, and abducens of the dog and of man; and the glossopharyngeal, accessory, and hypoglossal nerves of the dog, the cat and the rabbit. The nerves were obtained by lifting off the skull cap and following them distally from their cerebral origin by chipping away the base of the skull about their foramina of exit. The dissected specimens were laid on glass slides and prepared by different methods. Some were stained by the pyridine-silver method; others were placed in 50 per cent pyridine solution for seven days, washed, and then treated with silver nitrate, water, and pyrogallie acid as in the pyridine-silver method; others were stained by the Pa'-Weigert method and the osmic acid method. All were cut and mounted serially.

The oculomotor, trochlear, and abducent nerves form a natural group formerly described as purely motor and consisting of large and small myelinated axons, but now recognized as containing somatic afferent as well as efferent fibers. The nerves are described as communicating with the ophthalmic division of the trigeminal nerve and with the cavernous plexus of the sympathetic system.

¹ Contribution no. 40.

Gaskell ('89) studied the cranial nerves with especial reference to their relation to a typical spinal nerve. The oculomotor, trochlear and abducent nerves he described as pure efferent nerves. The oculomotor was composed of large and small myelinated fibers, in the dog 14.4 to 18 micra and 3 to 5 micra in diameter. The smaller fibers passed to the ciliary ganglion, the larger to the extrinsic muscles of the eye. The trochlear nerve consisted of large myelinated fibers, 14.4 to 18 micra in diameter, supplying the external oblique muscle, and small myelinated fibers 3.6 to 5.4 micra in diameter, of unknown function. The abducens consisted of large myelinated fibers 14.4 to 18 micra in diameter, and a few smaller ones, but contained no distinct group of small fibers as did the IIIrd and IVth nerves. In the roots of these nerves he saw fibrillar structures which he interpreted as the remains of ganglion cells present at an earlier stage of their development, and representing the afferent portion of the nerves.

Barratt ('98, '99, and '01) described the oculomotor nerve as composed of large and small myelinated fibers, 11 to 15 micra and 3 to 5 micra in diameter, in about the ratio of three to one. He found unmyelinated fibers also, "both in the fibrous sheath and in the main trunk; in the latter situation usually at the periphery." He did not find any communication with the cavernous plexus nor with the ophthalmic division of the trigeminal nerve. The trochlear nerve was composed of large and small myelinated fibers 12 to 19 micra and 4 micra in diameter, in the ratio of three to one. There were no unmyelinated fibers present. The abducent nerve he described as composed of large and small myelinated fibers, 11 to 17 and 3 to 6 micra in diameter. A few small twigs composed of small myelinated and unmyelinated fibers joined it 25 mm. from its superficial origin, and left it again 10 mm. distalward.

Carpenter ('06) studied the development of the oculomotor and abducent nerves in the chick. He found that the oculomotor nerve was composed of myelinated axons 3 to 15 micra in diameter. The trunk was composed of comparatively large axons, with a few scattered small axons, and a zone of small

axons at the periphery which passed into the ciliary ganglion. He found a communicating branch extending from the ophthalmic division of the trigeminal nerve to the ciliary nerve, but none to the undivided oculomotor nerve. He did not speak of a direct communication between the nerves studied and the sympathetic system, but referred to Jegorow's suggestion that a distribution of abducent fibers to the eyeball in birds might be accounted for by sympathetic fibers joining the abducent as it passed through the cavernous sinus.

Boughton ('06) found an almost regular increase in the number and size of myelinated fibers in the oculomotor nerve of the white rat and the cat at different ages. In rats of 730 days weighing 414 grams the large fibers averaged 8.1 micra in diameter, the small 4.3 micra. In cats of 2893 grams the large fibers averaged 13.5 micra in diameter, the small 7.2 micra.

Kopsch ('07) described the oculomotor nerve in man as composed of about 15 000 mostly large myelinated fibers grouped in a number of secondary bundles. In the roots between the fibers were isolated, branched, spherical nerve cells. The trochlear nerve he described as composed of about 1200 myelinated fibers, the abducent of about 2600 myelinated fibers. All three nerves received communicating branches from the carotid plexus of the sympathetic system, and from the ophthalmic nerve.

THE OCULOMOTOR, TROCHLEAR AND ABDUCENT NERVES

Specimens of the oculomotor nerve of the dog and of three human adults were studied in serial sections. Upon dissection at least two fine branches from the sympathetic system could be seen joining the nerve. Microscopically sections showed in each case clear pictures of a typical motor nerve (fig. 1) composed of large and small myelinated axons. In the human the ratio of large to small axons was about three to one; in the dog it varied between two to one and three to one. In osmic acid preparations the large myelinated fibers of the dog averaged 12 to 16 micra, the small 3 to 6 micra. Close to its peripheral origin the fibers of the nerve were grouped in one large bundle, with incomplete septa extending toward the center of the nerve. As

the nerve approached the cavernous sinus the septa became complete, and divided it into from five to eight fascicles of varying size. Nowhere along the course of the undivided nerve were unmyelinated fibers seen within its substance. In sections of the intra-cavernous portion of the nerve a few clusters of unmyelinated fibers were seen approaching it, but could not be followed into its substance. The characteristic grouping of

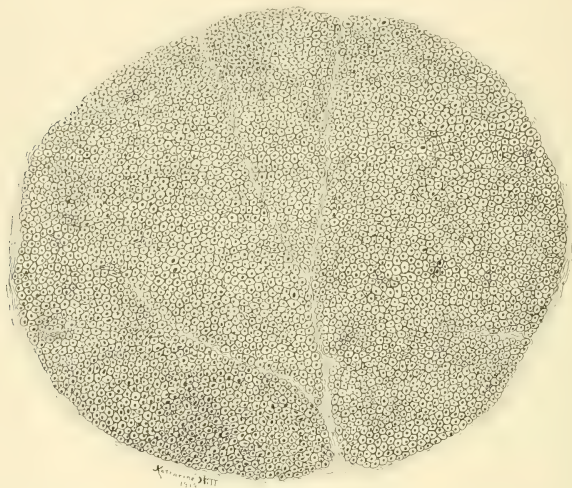


Fig. 1 Section of the oculomotor nerve of the dog. Pyridine-silver. Ocu. 0, Obj. 6.

small fibers at the periphery of the nerve, noted by Gaskell and Carpenter, could be seen most clearly in osmic acid preparations; but not all of the small fibers were grouped at the periphery, nor did they all leave the nerve to pass to the ciliary ganglion. The cellular structures in the roots of the nerves, mentioned by Gaskell and other observers, were not observed in our preparations, as even in the most proximal sections the roots had already united to form a common trunk.

Microscopic sections of the trochlear nerves of the dog closely resembled those of the oculomotor nerve; large and small myelinated fibers, 11 to 15 and 2 to 6 micra in diameter, in about the ratio of three to one, with a few fibers of intermediate size. No communicating branches from the cavernous plexus or the ophthalmic nerve were seen.

The abducent nerve of the dog near the brain stem consisted of a single group of large and small myelinated fibers, 11 to 15 and 3 to 6 micra in diameter, in about the ratio of three to one. Within the cavernous sinus the nerve was joined by a large bundle of sympathetic fibers, the majority of which formed an intimate union with three of the six or seven fascicles which made up the nerve at this point (fig. 2). More distally the main group of sympathetic fibers left the nerve to pursue an independent course.

The striking characteristic of these three nerves is their similarity in structure, both as to the size of the fibers and the proportion of large and small fibers. Gaskell and Carpenter noted the presence of small fibers in the oculomotor nerve, and considered the ciliary ganglion as their destination, though they did not say that all the small fibers entered the ganglion. Gaskell further noted the similarity in structure between the IIIrd and IVth nerves, but said the destination of the small fibers of the latter was as yet unknown. The VIth nerve he described as composed of large myelinated fibers "with a few smaller ones; with no sign of any distinct group of small fibers as in the IIIrd and IVth nerves." In our preparations all three nerves showed strikingly similar characteristics as to the size of the fibers and the ratio of the small and large fibers. The VIth nerve could not be distinguished from the other two by a diminished number of small myelinated fibers, but was characterized by the presence of unmyelinated fibers from the sympathetic system which came into intimate contact with the myelinated fibers and travelled distalward with them as far as the nerve was followed. The only clue to an explanation of this fact is Jegorow's suggestion that a distribution of fibers of the VIth nerve to the eyeball in the birds might be accounted for by the presence of sympa-

thetic fibers which joined the nerve as it passed through the cavernous sinus. No such communications from the sympathetic system were seen in connection with the IIIrd and IVth nerves. Branches of the cavernous plexus, which on gross dissection could be seen to join the IIIrd nerve, on microscopic



Fig. 2 Section of the abducent nerve of the dog, showing bundles of sympathetic fibers, *a*, Pyridine-silver. Ocu. 0, Obj. 6.

examination were seen to accompany the nerve for a short distance and again separate from it farther distalward, without entering into intimate contact with its fibers. No communication whatever between the IVth nerve and the sympathetic system was seen.

The unmyelinated fibers seen in connection with the IIIrd and VIth nerves nowhere showed the characteristic arrangement of the unmyelinated fibers seen in the spinal nerves and the vagus. In the IIIrd nerve the unmyelinated fibers remained grouped in the nerve sheath and did not enter its substance. More distally they separated from the nerve completely. The unmyelinated fibers which joined the VIth nerve and entered into intimate association with the myelinated fibers were not uniformly distributed throughout the nerve substance, but remained at the periphery, definitely grouped in two or three of the fascicles of the nerve. In no sections did these unmyelinated fibers from the sympathetic system appear evenly and uniformly distributed throughout the nerve substance as do the unmyelinated fibers in the spinal nerves and the vagus.

THE TRIGEMINAL NERVE

The intracranial portion of the trigeminal nerve of the dog and the cat, and of one human specimen was studied. Pyridine-silver sections showed large and small myelinated fibers, and in addition small numbers of unmyelinated fibers, appearing in largest numbers in two fascicles of the sensory portion of the nerve. No sympathetic fibers were seen joining the nerve within the cranium. In osmic acid preparations of the Vth nerve of the dog the large myelinated fibers measured 12 to 16 micra in diameter, the small 3 to 6 micra. In the cat the small fibers measured 4 to 7 micra, the large 12 to 16 micra, with occasional fibers 18 micra in diameter.

THE GLOSSOPHARYNGEAL, ACCESSORY AND HYPOGLOSSAL NERVES

The glossopharyngeal nerve is usually described as a mixed nerve, and is recognized, according to Herriek's classification, as containing both general and special visceral efferent fibers, and general and special visceral sensory fibers, as well as somatic fibers with sensory function. The accessory and hypoglossal nerves are pure motor nerves, the former containing general and special visceral fibers, the latter special somatic fibers.

Gaskell described the small myelinated fibers in the glosso-pharyngeal nerve as 1.8 to 3.6 micra in diameter, the large as not exceeding 10.8 micra. He found that large myelinated fibers were present in all the roots of the accessory nerve, but that the small fibers were confined to the bulbar and upper cervical roots. Barratt described the IXth nerve as composed chiefly of small myelinated fibers 4 micra in diameter. Kopsch described the IXth nerve as consisting of a motor and sensory portion, and

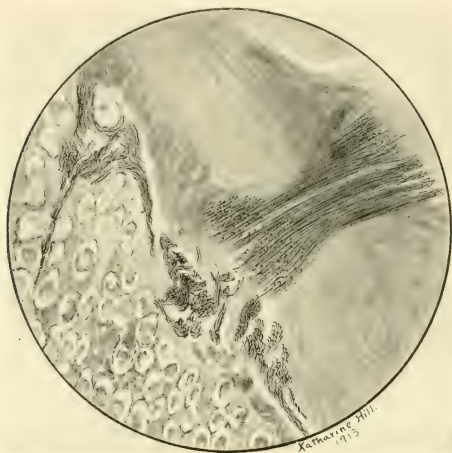


Fig. 3 From a section of the accessory nerve of the dog, showing a bundle of sympathetic fibers joining the nerve. Pyridine-silver. Ocu. 0, Obj. 8.

receiving sympathetic fibers from the superior cervical ganglion. He described the accessory nerve as arising in two parts, the *accessorius vagi*, which joined the vagus as the *ramus internus*; and the *accessorius spinalis*, which as the *ramus externus* received fibers from the jugular ganglion of the vagus. Chase and Ranson found numerous unmyelinated fibers in the roots of the vagus but thought the bulbar rootlets of the accessory nerve contained few if any unmyelinated fibers. Kopsch described the hypoglossal nerve as arising in 10 to 15 root bundles, and

forming anastomoses with the vagus, the upper three cervical nerves, and the superior cervical ganglion of the sympathetic system.

Pyridine-silver sections of the roots of the glossopharyngeal nerve showed large and small myelinated fibers, and a few

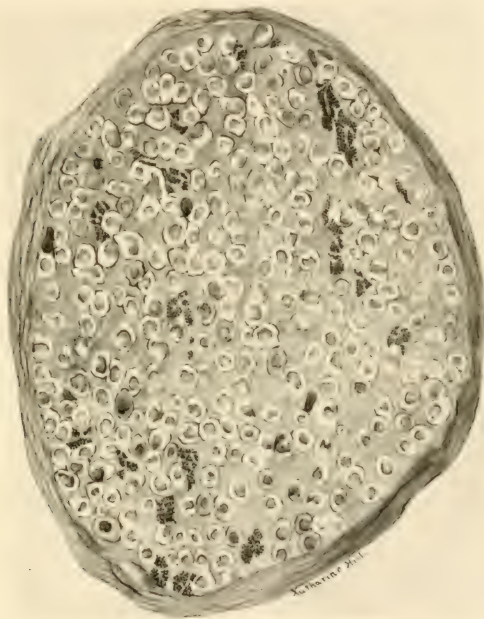


Fig. 4 Section of the accessory nerve of the dog, showing bundles of darkly stained unmyelinated fibers. Pyridine-silver. Ocu. 0, Obj. 8.

unmyelinated fibers. Sympathetic fibers could be followed into the trunk of the IXth nerve between its two ganglia. In the trunk of the nerve the small myelinated fibers outnumbered the large fibers nine to one. In osmic acid preparations the large fibers measured 9 to 12 micra in diameter, the small 3 to

5 micra. Sections of a pyridine-silver preparation of the IXth nerve of the cat, just proximal to the superior ganglion, showed few large myelinated fibers 12 to 15 micra in diameter, many small myelinated fibers, 3 to 6 micra, and a few unmyelinated fibers. Sections of the IXth nerve of the rabbit showed a similar picture. No connections with the sympathetic system distal to the petrous ganglion were seen in any preparations.

The bulbar rootlets of the accessory nerve are composed chiefly of small myelinated fibers 2 to 5 micra in diameter. The spinal



Fig. 5 From a section of the hypoglossal nerve of the dog, showing bundles of darkly stained unmyelinated fibers joining the nerve. Pyridine-silver. Ocu. 0, Obj. 8.

portion was composed of large and small myelinated fibers in about the ratio of five to one. The majority of the large fibers were 9 to 12 micra in diameter, with a few larger fibers of 14 to 16 micra; the small fibers 2 to 5 micra. In several preparations at the point of its separation from the vagus, a few unmyelinated fibers could be seen in the ramus externus, arranged in three or four groups. Bundles of sympathetic fibers could be traced for some distance in the sheath of the XIth nerve, finally entering it (fig. 3) and forming well marked clusters of fibers within its

substance (fig. 4). In Pal-Weigert preparations the bluish-black myelin rings were separated in places by small unstained areas, corresponding closely with the location of the unmyelinated fibers seen in pyridine-silver preparations. No essential differences were seen in the XIth nerve of the cat and rabbit. The large myelinated fibers of the spinal root of the accessory of the cat were the largest of all the fibers measured, some of them having a diameter of 18 micra.

The hypoglossal nerve of the dog showed a picture closely resembling that of the oculomotor nerve, large and small myelinated fibers in about the ratio of three to one, measuring 11 to 15 micra and 3 to 6 micra in diameter. No unmyelinated fibers were seen within the roots of the nerve, but slender bundles of sympathetic fibers could be followed as they approached the nerve, entered its sheath, and finally joined the nerve substance (fig. 5). The hypoglossal of the cat and rabbit showed similar pictures.

Nowhere did the unmyelinated fibers seen in sections of the XIth and XIIth appear evenly distributed among the myelinated fibers, as in the spinal nerves and the vagus; but always grouped in clusters, as in the VIth nerve, and in largest numbers near the periphery.

SUMMARY

1. Unmyelinated fibers are present in the Vth, VIth, IXth, XIth and XIIth cranial nerves. Those in the VIth, XIth and XIIth are probably all derived from the sympathetic system. In these nerves they have an arrangement characteristic of sympathetic fibers; they are grouped in clusters, and are most numerous near the periphery of the nerve.

2. The oculomotor and trochlear nerves are strikingly similar in composition; they are composed of large and small myelinated fibers, without any accessions or unmyelinated fibers from the sympathetic system.

3. The abducens is similar to the IIIrd and IVth nerves as regards its myelinated fibers, but in addition receives a large number of unmyelinated fibers from the sympathetic system.

some of which enter the nerve sheath and travel distalward with the myelinated fibers.

4. The accessory and hypoglossal nerves are composed of large and small myelinated fibers, and are similar in appearance and structure to the IIIrd and IVth nerves. Like the VIth nerve, they receive considerable numbers of unmyelinated fibers from the sympathetic system which can be followed to the termination of the nerves in the muse'es which they supply.

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THE BRAIN AND THE FRONTAL GLAND OF THE CASTES OF THE 'WHITE ANT,' *LEUCOTERMES FLAVIPES*, KOLLAR

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TWENTY-SIX FIGURES

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INTRODUCTION

This paper was undertaken with the idea of studying the finer structure of the brains of the different castes of the common termite or 'white ant,' and to compare them with the brains of the castes of true ants, the chief point of interest being that both termites and ants are social insects, living in communities

composed of many types of individuals, but differing greatly in their degree of specialization and in intelligence. As the work progressed it was found necessary to study the frontal gland in detail, and it is now my intention to follow this paper with a second, dealing with the development of the frontal gland and the differentiation of the different types of brains in the recently hatched nymphs.

The only form to be discussed in the present paper is the common termite, *Leucotermes flavipes*, Kol. As is well known, this insect is not an ant, but belongs to the order Isoptera, family Mesotermitidae, Holmgren ('11). The species, *flavipes*, was described by Kollar ('37); the genus *Leucotermes*, formerly included in the Linnaean genus *Termes*, was established by Silvestri ('01).

In the northeastern United States there is only this one genus and species of termites; in the southern states there is a second species, *L. virginicus*, with three different genera in the Gulf states, Snyder, and several other genera occur in California and the southwest, Heath ('03).

MATERIAL AND METHODS

My material was collected in Wellesley, Mass., in May, 1915, and June, 1916, beneath and within some old planks of wood. The fixatives used were Bouin's Fluid, and Gilson's Fluid. The former, however, is decidedly better for termite nervous tissue, and three hours in the fluid gives a good fixation. Whole mounts of all the heads were made by staining a long time, twenty-four to thirty-six hours, in Conklin's picro-haematoxylin, and then destaining for a day or longer in acid alcohol, clearing in cedar oil and mounting with the frontal side up. The heads of workers and of both types of nymphs, were embedded in hard paraffin, three to four hours, and were sectioned at a thickness of 6 μ . The frontal or horizontal plane proved the most satisfactory for sections of the head. In the case of the soldier, where the chitin of the head is very thick, the brain was first dissected out, under a dissecting lens, stained, embedded, and then sectioned. This was also done for the worker, in addition to sections of the entire head, the great hardness of the chitin making

the sections of this caste frequently imperfect. Whole mounts of the true adult and other brains were also made. It should be stated here that on account of the internal chitin of the tentorium, in addition to the chitin in the cuticula of the skin, the termite head is a difficult object to section. Only the prolonged time in paraffin has given good results. The sections were stained on the slide with (1) Ehrlich's acid haematoxylin and eosin, or (2) iron haematoxylin and orange G.

For the adult sexual forms, I am indebted to Mr. A. D. Hopkins and Mr. T. E. Snyder of the division of forest entomology of the U. S. Department of Agriculture, who have kindly furnished me with young and old adults of both the 'true' and the neotenic type with short wing pads, and with much other termite material that will be utilized later. I wish here to express my thanks to both Mr. Hopkins and Mr. Snyder for their kindness in sending me this material.

THE MEMBERS OF THE COLONY OF *L. FLAVIPES*

I. The adult castes and the young

The colony of *Leucotermes flavipes* is composed of many different kinds of individuals, some of which are adult or final stages, the castes, while others are merely the young, or the developmental stages of the castes, their presence, in some degree, depending upon the season of the year. Each caste contains both males and females, caste not corresponding with sex, as in the bees and ants.

The term adult is here used in the general and biological sense to denote an insect which has undergone its last molt, and which has, in general, acquired its final definitive form and structure. The phrase "in general" is inserted to cover the case of those older females whose abdomens have become greatly enlarged in the course of egg-laying. I am aware that some biologists would exclude from the category of adult stages the neotenic reproductive forms and the sterile workers and soldiers, on the ground that the former do not possess the outer bodily structure characteristic of the species, as seen in the 'true' adult,

TABLE 1
Adapted from Grassi

LEACOTERMES FLAVIPES KOL.

YOUNG OR DEVELOPMENTAL STAGES

ADULT OR FINAL STAGES

YOUNG OR DEVELOPMENTAL STAGES		ADULT OR FINAL STAGES
Eggs—Undifferentiated nymphs.....		Sex organs developed
{ Small headed nymphs (sex organs will develop)..... }	1. Nymphs of the first form, 6.5 to 7.5 mm. (long wing pads)	1. True adults. ♀ ♂, 6.5 to 7.5 mm. (long wings)
	2. Nymphs of the second form, 6.5 to 7.5 mm. (short wing pads)	2. Neotenic substitute forms, ♀ ♂, 6.5 to 7.5 mm. (short wing pads)
	3. Nymphs with no wing pads, ? mm.	3. Neotenic substitute forms. ♀ ♂ ? mm. (no wing pads)
Eggs—Undifferentiated nymphs.....		Sex organs not developed
{ Large headed nymphs (sex organs will not develop)..... }	4. Worker nymphs.	4. Workers. 5 to 6 mm. ♀ ♂ (wingless)
	5. Soldier nymphs.	5. Soldiers. 6 to 7 mm. ♀ ♂ (wingless)

and that the latter have undeveloped sex organs. One disadvantage of excluding these forms is that we are then left with no general term to denote the full grown stage, which after all is the original sense of the word adult.

The adult stages may be divided into two groups: (1) forms with functional and mature sex organs, the 'kings' and 'queens,' of which there are three different types, (a) kings and queens with long functional wings, the 'true' royal pair, (b) kings and queens with short wing pads, the neoteinic substitute pair with short wing pads; (c) kings and queens with no wing pads, the neoteinic substitute wingless pair, sometimes known as ergatoid or worker-like; (2) forms with undeveloped sex organs, (d) the 'workers,' (e) the 'soldiers,' making in all five adult castes. To these five types of adults, three more, representing phases of a later development, may be added, namely: the enormously distended fertilized egg-laying queens of the three queen types (table 1).

The young, or the developmental stages of the five adult castes, occur in many stages, the number of these differing with the caste. From the egg hatch forms which are said to be all alike and undifferentiated, Grassi, Snyder, and from these undifferentiated nymphs, 'larvae' of Snyder,¹ develop nymphs with large heads and nymphs with small heads. The large-headed nymphs develop "after a series of molts and quiescent stages of comparatively short duration," the whole process requiring less than one year, Snyder ('16), into the sterile workers and soldiers. The small-headed nymphs undergo a more complex development, which "apparently requires two seasons," and develop into: (1) nymphs of the 'first form,' with long wing pads, which become the true royal pair, the sexual adults with long wings, (2) nymphs of the 'second form,' with short wing pads, which become the substitute sexual forms with short wing pads, (3) nymphs without wing pads, whose development is not fully understood, and which become the substitute wingless sexual adults.

¹ The term nymph is used in this paper to denote any developmental stage of an insect with incomplete metamorphosis, whether the form possesses wing pads or not. Snyder, in accordance with the older authors, applies the term 'larva' to the younger nymphs which possess rudiments of wing pads that can only be distinguished with magnification. He uses the term nymph to designate forms with wing pads that can be distinguished with the naked eye.

2. *Distinguishing characters of the five adult castes and of three types of nymphs*

Before beginning the discussion and comparison of the brains of the different forms of *L. flavipes* some descriptions of the types themselves and the means of distinguishing them may be of interest.

Forms with sex organs developed in the adult. 1. NYMPHS. Nymphs of the first form, with long wing pads. These nymphs are most numerous in the early spring before the transformation into the adult has taken place. According to Snyder they attain a length of 7.5 mm. before the last molt, but they may be distinguished while much shorter. The color is creamy white, with a pale pink or rose pigmentation of the compound eyes. The hind wing pads extend back as far as the fourth abdominal segment.

Nymphs of the second form, with short wing pads. These nymphs are found at the same time and in about equal numbers with the long-winged nymphs. They likewise attain a length of 7.5 mm. before the last molt. They may be readily distinguished from the 'first form' by the short rudimentary wing pads which hardly reach the second abdominal segment. The color is creamy white and the compound eyes show no trace of pigment.

Nymphs with no wing pads. I have never found these nymphs and therefore quote from Snyder, "Another type of substitute or neoteinic reproductive form, which greatly resembles the worker ('ergatoid') is developed from young larvae of the sexed forms."

2. ADULTS. The true royal pair, with long wings. The true winged adults are found in the nest or in the air in late spring and early summer. The head and body are dark brown, of about the same size as the nymphs of the first form, the long filmy wings are nearly twice the length of the body. The compound eyes are black surrounded by a lighter rim of unpigmented skin. The two lateral ocelli are visible as small light spots in front of the compound eyes and behind the antennae. The opening of the frontal or fontanel gland lies in the median line of the frontal surface of the head.

The egg-laying true queen. The old egg-laying true queens are always found within the nest, often in large 'queen cells' or chambers, and they may attain a length of 14.5 mm., Snyder ('16). The head and thorax are of normal size and brown in color, the abdomen is enormously distended, and is straw-colored with intersegmental bars of brown. The broken off stubs of the long wings remain attached to the thorax.

The neotenic substitute pair with short wing pads. This pair is found within the nest and is similar in length to the nymphs of the second form. The body color is light brownish and the compound eyes are brown. The wing pads are now merely short, transparent chitinous plates.

The egg-laying substitute queen with short wing pads. This old substitute queen with short wing pads attains a length of 9 mm., Snyder ('16). The abdomen is distended and is creamy white with darker chitinous bars, the head and thorax are of normal size. The compound eyes are brown.²

The neotenic substitute wingless pair. As I have never seen this form, I will again quote from Snyder ('16, p. 7), "Of a pale yellowish or grayish color, and having no wing pads (fig. 3; fig. 4c), individuals of this larval supplementary reproductive form are apparently blind and never leave the parent colony, except by underground tunnels."

The egg-laying substitute wingless queen. The old egg-laying substitute wingless queen attains a length of 9 mm. and has a distended abdomen, Snyder ('16).

Forms with sex organs not developed in the adult. 1. The workers. Workers are found in termite colonies throughout the year. The smallest of all the castes, the body measures only 5 mm. in length. The head is considerably broader from side to side than that of the nymphs. The abdomen is shorter, softer, and covered with a more transparent skin. The color of the abdomen is usually grayish, owing to particles of wood in the alimentary canal seen through the skin. No eyes are visible in

² The above descriptions of the enlarged egg-laying queens are based upon the alcoholic specimens sent to me by Mr. A. D. Hopkins and Mr. T. E. Snyder of the U. S. Dept. of Agriculture.

the worker until after staining, when small rudimentary compound eyes may be distinguished. No ocelli are present.

2. The soldiers. Soldiers are found in the colony throughout the year. The entire body measures 6 to 7 mm. in length, but the head is much longer and the abdomen shorter than that of the worker. No eyes can be seen until after the head is stained, when rudimentary compound eyes even smaller than those of the worker become visible. The opening of the frontal gland is in the median line of the frontal surface of the head.

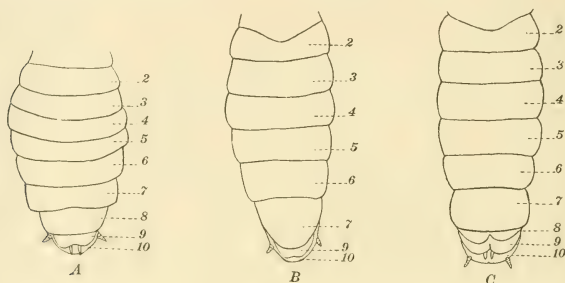


Fig. 1 The abdomens of three individuals seen from the ventral surface. A, adult male; B, adult female; C, female nymph with long wing pads; 2 to 10, 2nd to 10th abdominal segments. Obj. 32, oc. 6, reduced one-half.

III. Means of distinguishing the sexes

The only differentiation between the two sexes in any caste of *L. flavipes* is in the size of the abdomen, and in the relative size and arrangement of the sternites, i.e. the ventral parts, of the posterior abdominal segments.

The sex of any termite may be distinguished by the size and the shape of the seventh and eighth abdominal sternites.³

In the male viewed from the ventral surface (fig. 1, A) the seventh and eighth abdominal segments measure about the same in length, i.e., in an antero-posterior direction, and the seventh

³ The ventral surface of the first abdominal segment is entirely covered by the metathorax, so that the actual seventh segment is apparently the sixth.

segment is shorter than in the female (*B*). The posterior margin of the ninth segment is concave and two slender cerci are present on the lateral surfaces of the tenth segment.

In the adult female (fig. 1, *B*) the seventh segment is very long, with a rounded posterior edge, and completely covers the eighth and part of the ninth segment. Jointed cerci are borne on the sides of the tenth segment.

In all female nymphs (fig. 1, *C*) and also in the female workers and soldiers, the seventh segment, although long, does not completely cover the small indented eighth segment. Cerci similar to those of the male are borne on the median surface of the ninth segment and also on the lateral surfaces of the tenth segment.

GENERAL ANATOMY OF THE TERMITE BRAIN

The present paper will discuss the brains of the nymphs of the first and second forms, the true sexual adults, and the workers and soldiers.

It is generally thought today that the insect brain includes six regions, corresponding to the embryonic segments of the head, three of which are supraesophageal, namely: the protocerebrum, the deutocerebrum, and the tritocerebrum, and three subesophageal, the mandibular, the maxillary, and the labial ganglia. Holmgren ('09) finds these six regions in the termite brain and discusses at some length the origin of the labrofrontal nerves, which he decides is in the tritocerebrum. It need only be remarked here that the same six regions may be distinguished in *Leucotermes*, and that in agreement with Holmgren I find the labrofrontal nerves arising from the tritocerebrum. Table 2 names the regions and the parts that are present in the brain of *L. flavipes*.

Figure 2 is a diagram of the brain of *L. flavipes*, as seen from the frontal surface. The supraesophageal ganglion is drawn from an entire mount of a brain dissected out from the head of a nymph with long wing pads; the ventral connectives, *v.c.*, and

TABLE 2

The parts of the brain

I. The supraesophageal ganglion.

A. Protocerebrum

The protocerebral lobes

The protocerebral commissures { anterior, dorsal
posterior, dorsalThe mushroom bodies { anterior roots
central body roots
posterior roots

The central body

The optic lobes

B. Deutocerebrum

The antennal lobes

C. Tritocerebrum

The tritocerebral lobes

The tritocerebral commissure (subesophageal)

II. The subesophageal ganglion

The ventral connectives

The mandibular }
The maxillary } ganglion
The labial }*The nerves of the head*

I. A. The optic nerves

The ocellar nerves

The fontanel nerve

B. The antennal nerves

C. The labrofrontal nerves to { the nerve to the protocerebrum
the frontal ganglion { the labral nerves
the recurrent nerve

II. The mandibular nerves

The maxillary nerves

The labial nerves

the subesophageal ganglion, *sb.g.*, are drawn from sections of the same form.⁴

⁴ The heads of all termites are held in a somewhat slanting position, making a large obtuse angle with the long axis of the body, so that the morphologically dorsal surface of the head has become frontal or anterior. The slant of the head is much less in termites than in ants, and in the termite soldier the head is almost horizontal, the so-called frontal surface being practically dorsal, but for the sake of clearness the same terms of direction will be applied to all the castes of termites. In describing the entire heads the terms anterior and posterior imply toward and away from the frontal surface, in the same sense that Hesse (1901 b) uses the terms rostral and caudal; in like manner dorsal and ventral imply toward or away from the vertex of the head.

The insect brain forms a ring of nerve tissue which encircles the esophagus and which is composed of the supraesophageal ganglion, the ventral connectives, and the subesophageal ganglion.

The three parts of the supraesophageal ganglion, the protocerebrum, the deutocerebrum, and the tritocerebrum, are merged into a single mass which constitutes the principal part of the brain. To the protocerebrum belong the protocerebral lobes, *p.l.*, the optic lobes, *o.l.*, connected by the fibers of the optic

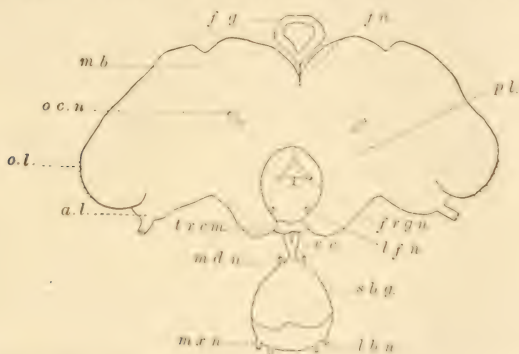


Fig. 2 Diagram of the brain of a nymph with long wing pads, as if seen in frontal optical section. *A.l.*, antennary lobe; *f.g.*, frontal gland; *f.n.*, fontanel nerve; *fr.g.n.*, frontal ganglion; *l.f.n.*, labrofrontal nerve; *lb.n.*, labial nerve; *m.b.*, mushroom body; *md.n.*, mandibular nerve; *mx.n.*, maxillary nerve; *oc.n.*, ocellar nerve; *o.l.*, optic lobe; *p.l.*, protocerebral lobe; *sb.g.*, subesophageal ganglion; *tr.cm.*, tritocerebral commissure; *v.c.*, ventral connective.

nerves with the compound eyes, the two mushroom bodies, *m.b.*, the ocellar nerves, *oc.n.*, connected with the two lateral ocelli, and the fontanel nerve, *f.n.*, connected with the frontal gland, *f.g.* To the deutocerebrum belong the antennal lobes, *a.l.*, and the antennal nerves of the antennae. The tritocerebrum consists of the tritocerebral lobes, and the tritocerebral commissure, *tr.cm.*, beneath the esophagus. The labrofrontal nerves, *l.f.n.*, or the tritocerebral nerves, arise on the inner, median surfaces of the tritocerebral lobes, and running upward and forward unite in the frontal ganglion, *fr.g.n.*, which lies anterior to the main

part of the brain and directly above the mouth opening. The connection of the frontal ganglion and the labrofrontal nerves is not shown in the diagram nor is the recurrent nerve which runs backward from the frontal ganglion.

The delicate unpaired nerve from the dorsal surface of the frontal ganglion to the protocerebral lobes, and the labral nerves from the ventral surface of the frontal ganglion, are figured but not labeled. Posterior to the tritocerebral commissure the two slender ventral connectives, *v.c.*, run first backward (which cannot be shown in this diagram), then downward, and unite to form the large subesophageal ganglion, a single mass, consisting of the fused mandibular, maxillary, and labial ganglia, and from which arise the mandibular, maxillary, and labial nerves. Posterior to this ganglion the thoracic connectives, not shown in the figure, pass upward and then backward into the thorax.

THE FINER STRUCTURE OF THE BRAIN

I. The brain sheath

The entire brain is surrounded by a membranous sheath composed of a single layer of cells resembling mesenchym cells, their fibrous processes making a continuous double walled membrane between which the cell bodies and the nuclei are situated.

II. The protocerebral lobes

The protocerebral lobes (fig. 2, *p.l.*) form the central part of the supraesophageal ganglion; they are continuous on their dorsal surface with the mushroom bodies, on their lateral surfaces with the optic lobes, and on their ventral surface with the antennal lobes, and are also connected by a slender nerve with the frontal ganglion, *fr.gn.*, which lies anterior and somewhat ventral to the protocerebrum. The ocellar nerves, from the lateral ocelli, and the fontanel nerve, from the frontal gland, also enter the protocerebral lobes, and will be considered in more detail under those headings. Like other parts of the brain the protocerebral lobes consist of a central fibrous core and an outer investing layer of nerve cells. Most of these nerve cells are small

with round nuclei and a round cell body which appears as a mere rim of cytoplasm (fig. 3, *A, B*), but in the median dorsal region, the intercerebral region of Haller, and here and there on the ventral surface, the cells are larger with pear-shaped cell bodies (fig. 3, *C, D*). The smallest nerve cells of the protocerebral lobes are, however, almost twice as large as the adjacent nerve cells belonging to the mushroom bodies (fig. 3, *E*) and to the optic lobes.

In a series of frontal sections, beginning with the frontal or anterior surface (figs. 13 to 20) it will be seen that the proto-



Fig. 3 Nerve cells from the protocerebral lobes and the mushroom body. *A*, small cells, from the lateral region of the protocerebral lobes; *B*, medium sized cells, from the same region; *C*, large cells, from the median ventral region of the protocerebral lobes; *D*, large cells, from the intercerebral region; *E*, cells from the mushroom body. Homog. immers. 1.8 mm., oc. 6.

cerebral lobes are at first entire (fig. 13), then, farther back in the series (fig. 14) the two great anterior roots of the mushroom bodies penetrate deep into the fibrous core of the protocerebral lobes, dividing them into a median and two lateral portions, and this division is further continued by the stalks of the mushroom bodies (figs. 15, 16).

a. The protocerebral commissures. About the middle of the protocerebral lobes, in the plane of the central body (fig. 15) the lateral and median parts of the lobes are again connected by a stout fiber tract passing from side to side beneath the central body and connected also with the antennal lobes. This tract or commissure is homologous with the so called 'ventral com-

missure' of the hymenoptera and other insects. The lateral portions of the protocerebral lobes are also connected by two dorsal protocerebral commissures, the anterior, a delicate strand of fibers, lying above the central body (figs. 15, 16, *a.cm.*) the posterior, on the same level as the first, lying immediately posterior to the central body, fig. 18, *p.cm.* These two commissures are homologous with the two dorsal commissures found in ants, and it is interesting to note that the posterior one, the 'Brücke' of the older authors, has the same characteristic wide inverted \cap shape.

In figures 15 to 18, the connection of the optic lobes with the protocerebral lobes may be seen.

Figures 19 to 20 show the relation between the protocerebral lobes, the deutocerebrum or antennary lobes, and the tritocerebrum.

III. The mushroom bodies

The two mushroom bodies, or the globuli, as Holmgren terms them, are now generally considered the chief centers of intelligence in insects. They appear, in frontal views of the head (figs. 8 to 12, *m.b.*), as two slightly grooved projections from the dorsal surface of the brain, the frontal gland occupying the space between them. Each mushroom body consists typically of two lobes, which in the termites are not distinctly separated but form one continuous mass of tissue, the lobes being indicated on the exterior of the brain merely by a slight groove or depression of the surface. In sections (figs. 15 to 18) it may be seen that the mushroom bodies are composed of an outer nerve cell layer and an inner fibrous portion which forms the cups or calyces, the stalks, and the three pairs of roots.

a. The nerve cells of the mushroom bodies. The nerve cells of the mushroom bodies are all of the same size and are among the smallest cells of the brain (fig. 3, *E*). They have a round or oval nucleus and so small an enveloping layer of cytoplasm that it cannot be distinguished, even with the immersion lens, except at the distal end where the axon is given off. The chromatin masses of the nucleus are all about the same size, forming a

peculiar characteristic pattern by which these cells may be recognized.

Although the nerve cells are of similar size throughout the mushroom bodies, they are differentiated into groups, or zones, according to their position. In the center of each cup or calyx, as seen in section (fig. 4), lies an oval mass of cells, *I*, which is homologous in position with the central oval mass of large cells found in the bees, Jonescu ('09), and in the ants, Pietschker ('11), Thompson ('12), and which I have termed Group I. On each side of Group I, are broad wedge-shaped masses of cells (fig. 4,

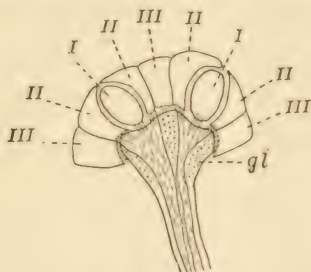


Fig. 4 Diagram of a mushroom body, showing the nerve cell layer divided into groups, the calyces, and the beginning of the stalk. *I*, cell group I; *II*, cell group II; *III*, cell group III; *gl.*, glia cells.

II), which occupy most of the dorsal surface of each lobe and whose inner margins overlap and enclose the central group I. These masses, which appear separate in sections, form a continuous zone if seen in surface view, and are homologous with Group II of ants. Again, in each lobe, on each side of Group II lie smaller masses of cells which form the lateral surfaces of the lobes (fig. 4, *III*). There are only three, instead of four, of these cell masses, because the inner lateral surfaces of the two lobes are in contact and their cells are continuous. These groups are equivalent in position to Groups III and IV of the ants.

b. The fibrous core of the mushroom bodies. The cups or calyces of the inner and outer lobes of the mushroom bodies are composed (1) of converging bundles of fibers, the axons of the three

zones of nerve cells just described, and (2) of masses of glia cells (fig. 4, *gl.*) resembling the 'glomeruli' of the antennal lobes, which envelop the fiber bundles on all sides and unite the calyces of the two lobes into one continuous whole.

The stalks of the mushroom bodies are the continuations downward and inward of the fiber bundles of each lobe. These lie side by side, the fibers from the inner lobe on the median side of those from the outer lobe, and for a short distance they remain distinct. The two stalks, surrounded by a delicate sheath, penetrate deep down into the fibrous core of the protocerebral lobes and then run forward. There is no 'decussation' of fibers such as is seen in the hymenoptera. In the same frontal plane with the central body (fig. 15) the distal ends of the stalks lie beneath the central body, and at this point each stalk gives rise to three roots, the anterior, the central body, and the posterior roots. The central body roots, which are the shortest, pass upward and directly into the central body (fig. 16, *c.b.r.*). The anterior roots which are the longest, bend sharply upward and forward, making an elbow with the stalks from which they have arisen (fig. 14, *a.r.m.b.*, *m.b.s.*). In sections that are farther forward in the head, the anterior roots may be seen as two great bundles of fibers curving latero-dorsally and dividing the protocerebral lobes in the manner already described. On reaching the dorso-lateral margins of the protocerebral core, the anterior roots curve in a posterior direction and are seen in sections as two detached masses on the outer sides of the mushroom bodies (figs. 15, 16, *a.r.m.b.*), then (fig. 14) returning again forward, the narrow distal ends turn downward and terminate among the nerve cells of the anterior part of the protocerebral lobes.

The posterior roots branch off from the stalks together with the central body roots, and, after the latter have passed into the ventral surface of the central body, pass dorsalward, posterior to the central body and to the posterior dorsal commissure, there expanding into two large and very prominent lobes which nearly fill the intercerebral region (figs. 19, 20, 23, 26, *p.r.m.b.*). The ventral part of these lobes or roots is connected with the protocerebral fibrous core, the dorsal part, however, projects back-

ward for some distance, evidently giving fibers to and receiving fibers from the posterior cells of the mushroom bodies, and then ending in about the same plane with the beginning of the trito-cerebral lobes.

c. Comparison of the mushroom bodies of the different castes of L. flavipes. The mushroom bodies differ little in the different castes except in size, and even in this respect there is not a very great dissimilarity (figs. 8 to 12).

The worker has the largest mushroom bodies, largest by actual measurement and in the estimated number of nerve cells; the nymphs of the first and second forms have mushroom bodies about similar in size, but slightly smaller than those of the worker; the soldier has the smallest mushroom bodies of any caste. The cells of the mushroom bodies of the worker and soldier penetrate into the intercerebral region (figs. 22, 25), but merely border upon it in those of the nymphs of the first and second forms.

My only true adult material, as already stated, was alcoholic and much of it seemed to have shrunk. It will be noted that in the true adult brain outlined in figure 9 the mushroom bodies are farther apart than in the nymph of the first form from which this adult has developed (fig. 8) and the frontal gland has increased in size. Some other better preserved true adult brains are considerably larger than the brain of a nymph of the first form, and the mushroom bodies are likewise larger.

Holmgren ('09) emphasizes the great deterioration of the brain tissue which he observes in the older enlarged and egg-laying queens. My material has been preserved in alcohol and is not the best for finer study, but the examination of several old and enlarged queens leads me to believe that in *L. flavipes* this deterioration of the brain has not taken place.

d. Comparison of the mushroom bodies of termites and hymenoptera. A comparison of the *Leucotermes* mushroom body with those of ants and bees shows, as one would naturally expect, that the termite mushroom body is much more simple and primitive. This primitive condition is apparent in the small and uniform size of all the nerve cells, especially in the cell

group I; in the presence of three zones of cells instead of the four found in ants; in the incomplete differentiation of the two lobes, whose cells are not separated by a deep furrow, as in ants, and whose two cups or calyces are completely fused by intervening masses of glia cells; in the shallowness of the cups; finally, in the smaller size of the entire mushroom bodies and their slight differentiation in the different castes.

IV. The central body

The central body is situated in the central frontal plane of the protocerebral lobes, embedded in their fibrous core, directly beneath the intercerebral region. In form as well as in position the central body of *L. flavipes* resembles that of bees and ants. It has two parts (fig. 15, *c.b.*), a curved dorsal portion, composed of fiber bundles that are radially arranged with intervening spaces, and a flatter ventral part, also fibrous. No nerve cells are found within the central body, as in ants, where small nerve cells occupy the spaces between the radial bundles of fibers, but a few scattered nerve cells are occasionally found along the outer surface.

The structure of the central body is the same in all the castes, but the size varies with the size of the different brains (figs. 15, 22, 25).

Lying beneath the central body are two small round bodies which were formerly known as the "tubercles of the central body" and also as 'ocellar glomeruli.' I have shown, Thompson ('12, '14), that in ants and in the bumble bee these previously misinterpreted bodies are the posterior roots of the mushroom bodies. In *L. flavipes* these rounded bodies are in connection with the central body roots of the mushroom bodies (figs. 15, 16, *c.b.r.*), which, it will be remembered, arise from the distal ends of the stalks in close proximity to the posterior roots.

V. Ocelli and ocellar nerves

Two simple eyes or ocelli are present in the adults and nymphs of the sexual forms, and are situated on the lateral surfaces of the head, in front of the compound eyes and behind the antennae.

In the head of the true adult (fig. 9, *o.c.*) the ocelli may be readily seen as small colorless spots which stand out sharply from the surrounding brown skin, but in the heads of nymphs (figs. 8, 10), whose skin is not pigmented, the ocelli are not visible except in sections.

The ocelli are of a very simple and primitive type, Hesse ('01 *b*), without a lens and with little or no pigment. The outer surface of the hypodermis above the ocelli is slightly convex, the inner surface is very concave, the latter caused by the sudden thinning of the inner cuticula, and in this concavity the bulb-like ocelli are situated. In general contour the ocelli resemble the tactile buds found in the skin of *Amblystoma punctatum*. The visual cells are long slender and curving, with spaces between their bases. As to the finer intracellular structure, I am not prepared to make a statement at this time. I shall also leave for further study the question whether the distal ends of the visual cells lie between the hypodermal cells, a primitive position, according to Hesse, or wholly, and secondarily, beneath them; although the first view would seem to be upheld by my present material.

In a frontal section of the anterior part of the brain of a nymph with long wing pads, the ocelli (fig. 13, *oc.*) may be seen on each side of the section, just beneath the hypodermis, and covered by a thinner layer of cuticula, the inner cuticula, *i.cu.*, being absent at this point. From the ocelli the slender ocellar nerves, lying just outside the brain sheath, run in toward the median line and enter the nerve cell layer of the protocerebral lobes just above the anterior roots of the mushroom bodies. Passing down into the intercerebral region, the ocellar nerves (figs. 15 to 18, *oc.n.*) run backward and finally enter the dorsal surface of the protocerebral lobes just behind the posterior dorsal commissure and in the same frontal section as the fontanel nerve from the frontal gland (fig. 19, *oc.n.*, *f.n.*). At no point along their entire length do the ocellar nerves expand into ocellar lobes such as are present in the bees and ants. After my first cursory examination of the brain sections of *L. flavipes*, I was inclined to assign the rôle of ocellar lobes to the large lobes,

p.r.m.b. seen in figures 19, 23, 26, on the ground that these lobes occupy a position similar to that of the ocellar lobes of ants and are in the neighborhood of the entering ocellar nerves. A very careful examination of a large number of sections, however, proved that there is no connection between the ocellar nerves and these adjacent lobes, and that there is direct connection between the mushroom body stalks, the protocerebral lobes, and the lobes in question, which are, as shown in a preceding section, the posterior roots of the mushroom bodies.

VI. *The optic lobes*

The optic lobes are situated on the lateral surfaces of the protocerebrum and are present in all the castes of *L. flavipes*, although they are well developed only in the sexual forms (figs. 8 to 12, *o.l.*). They are continuous with the protocerebral lobes and consist of an outer layer of very small nerve cells, similar in size to those of the mushroom bodies, and an inner fibrous portion that is subdivided into the outer, middle, and inner fiber masses, and the outer and inner crossings of fibers found in the typical insect brain, Berlese ('09).

The optic lobes of the nymph with long wing pads are the largest and most highly developed of any caste studied. The outer fiber masses (fig. 17, *o.f.*) are slender and elongated in a dorso-ventral direction. Nerve fibers pass in to them from the compound eyes, and continue inward, as the outer crossing, to the middle fiber mass, *m.f.*, the largest of the three masses. Fibers again cross between the middle and the inner fiber masses, *i.f.*, making the inner crossing. The small inner fiber masses are directly continuous with the fibrous core of the protocerebral lobes.

In the nymph with short wing pads (fig. 10) the optic lobes and the compound eyes are slightly smaller than in the nymph just described, but the relative size and arrangement of the parts is the same.

In the worker, although the optic lobes are very much reduced in size, they are readily seen in surface views (fig. 11) as small

projections from the lateral surfaces of the brain, and a slender optic nerve passes from each greatly reduced compound eye to the optic lobe. In a frontal section of the worker brain (fig. 25) the small inner, middle, and outer fiber masses may be distinguished in the optic lobes, and also the fibers of the distal part of the optic nerve. By carefully following the optic nerve in subsequent sections it may be traced to the vestigial compound eye.

The condition of the compound eyes evidently varies considerably in different termites. Holmgren ('09), describing the optic lobes in the worker of *Eutermes*, writes as follows:

Die Reduktion der Facettaugen hat eine entsprechende Reduktion der Sehganglien mitgeführt. Die Schnerven sind vielleicht vorhanden aber enthalten nur wenige Nervenfädchen und von den bei den Geschlechtstieren, besonders den jungen, so wohlentwickelten Sehganglien kann man gar nichts entdecken. Die Seiten des Protoocerebralganglions sind somit kreisförmig abgerundet.

The optic lobes of the soldier, like those of the worker, appear as small rounded projections on the lateral surfaces of the protocerebrum (fig. 12, *o.l.*), with optic nerves extending to the still smaller vestigial compound eyes, *c.e.* On account of the very hard chitin of the soldier's head no attempt was made to section the entire head. The brain was dissected out, under a dissecting microscope, stained, embedded, and then cut in frontal sections. A section through the optic lobes (fig. 22, *o.l.*) shows that the inner, middle, and outer fiber masses, although small, are clearly distinguishable, and that fibers enter the outer fiber mass from the optic nerve.

Holmgren ('09) states of the soldiers of *Eutermes*:

Die Soldaten sind ja blind und deshalb sind die Sehganglien vollständig verschwunden. Man kann von denselben kein Spur entdecken, obwohl ein sehr dünner n. opticus meistens vorhanden ist. Dieser fungiert aber als Hautnerv. Zufolge des Fehlens der Sehganglien sind die Seiten des Protoocerebrums beinahe kreisrund.

The optic lobes of the true adult have not been studied in sections.

VII. *The antennal lobes*

The antennal lobes compose the deutocerebrum, or the second brain segment, and are continuous with the anterior and ventral part of the protocerebrum (fig. 2, *a.l.*). In form they are elongated masses that continue into the antennae as the antennal or olfactory nerves. On their inner lateral surfaces the antennal lobes are continuous with the third brain segment, the tritocerebrum.

In sections it may be seen that the antennal lobes have an outer nerve cell layer containing both large and small cells, and an inner fibrous core that contains scattered masses of glia cells, the so-called 'glomeruli.'

The relative size of the antennal lobes differs very slightly in the different castes of *L. flavipes* (figs. 8 to 12). The antennal lobes are largest in the nymphs with long wing pads and in the worker, but are smaller in the nymph with short wing pads and in the soldier. Holmgren states that these lobes are much larger in the worker of *Eutermes* than in the other castes: "die Deutocerebralganglien der Arbeiter sind verhältnissmässig grösser und enthalten eine auch absolut bedeutend grössere Zahl Ganglienzellen als bei den Geschlechtsindividuen."

VIII. *The tritocerebral lobes and the tritocerebral commissure*

The tritocerebral lobes are small lobes extending ventrally along the sides of the esophagus and continuous with the inner, lateral, surfaces of the antennal lobes (fig. 20). From the inner median surfaces of the tritocerebral lobes arise a pair of nerves that run forward alongside of the esophagus and unite in the frontal ganglion; these are the tritocerebral or labrofrontal nerves (fig. 2, *l.f.n.*). Beneath the esophagus the tritocerebral lobes unite in the slender tritocerebral commissure (fig. 2, *tr.cm.*), which is almost devoid of any investing nerve cells.

IX. *The frontal ganglion*

The frontal ganglion is a small mass of nerve tissue situated just beneath the clypeus, the sclerite of the head to which the labrum is attached, and above the mouth opening, somewhat antero-ventral to the brain (figs. 2, 8, *fr.gn.*).

In sections (figs. 13, 21, 24) the frontal ganglion is rather triangular in appearance, the apex composed of nerve cells and the base of nerve fibers. The nerves connected with the frontal ganglion are (1) the paired tritocerebral or labrofrontal nerves, *l.f.n.*, (2) the paired labral nerves, *la.n.*, (3) an unpaired nerve to the protocerebrum, (4) the unpaired recurrent nerve, *r.n.*

The tritocerebral or labrofrontal nerves, *l.f.n.*, arise from the tritocerebrum as described under that heading, and their anterior ends are connected with the latero-ventral surfaces of the frontal ganglion.

From the median ventral surface of the frontal ganglion a bundle of nerve fibers emerges that runs first ventralward for a short distance and then divides into delicate right and left branches that pass to right and left through some muscle fibers in the clypeus, continuing down and forward into the labrum as two delicate but distinct nerves. Those branches are distributed throughout the labrum. I have termed these nerves the labral nerves. In this I am apparently not in agreement with Holmgren ('09) who does not describe the labral nerves as arising from the frontal ganglion but speaks as though the labrum were innervated directly from branches of the labrofrontal nerves, which is not the case in *L. flavipes*. In all the sections which I have examined the nerves of the labrum have no direct connection with the labrofrontal nerves but arise indirectly, from the base of the frontal ganglion as described above. To quote Holmgren:

Ganglion frontale liegt unmittelbar vor dem oberen Schlundganglion und ist mit diesem durch einen unpaaren Nervenfasern, der im Protocerebrum eintritt, verbunden. Vor dem Ganglion frontale gehen nach vorn Nerven aus, welche einige clypealen Muskeln innervieren.

An den Seiten des Ganglion frontale kommen die tritocerebralen Connective ein, und hinten läuft der nervus recurrens aus, um oberhalb des Darmes sich nach hinten zu begeben.

Aus Tritocerebrum werden folgende Teile innerviert:

- 1) Die ganze oben beschriebene Labralmuskulatur von n. labrofrontalis
- 2) Die Labraldrüsen von n. labrofrontalis.

In agreement with Holmgren I find a very delicate unpaired nerve running between the dorsal apex of the frontal ganglion

and the ventral surface of the protocerebral lobes, also the unpaired recurrent nerve which arises from the posterior surface of the frontal ganglion. This latter nerve (figs. 14 to 26, *r.n.*) runs backward above the esophagus into the thorax and at a short distance posterior to the supraesophageal ganglion expands into the so-called 'esophageal ganglion.'

X. The ventral connectives and the subesophageal ganglion

The two slender ventral connectives (fig. 2, *v.c.*) arise from the posterior surface of the tritocerebral commissure. They continue backward side by side, their inner surfaces touching, beneath the esophagus and above the broad tentorial band of chitin that is present in the interior of the head. They become still smaller, and pass down through the tentorial aperture, finally merging into the dorsal surface of the subesophageal ganglion.

The subesophageal ganglion (fig. 2, *sb.g.*) is large and consists of a fibrous core and a thick investing layer of nerve cells on the ventral surface. From the anterior end arise the mandibular nerves, *md.n.*, from the posterior end, the maxillary, *mx.n.*, and the labial, *lb.n.*, nerves. The branches of these nerves, described by Holmgren, have not been traced. Immediately behind the point of exit of the labial nerves the subesophageal ganglion ends, or rather is continued into the thoracic connectives, which pass upward and out of the head through the great tentorial aperture.

XI. The frontal or fontanel gland and the fontanel nerve

The frontal or fontanel gland of the termites has long been an object of much interest and speculation. It is situated in the median line of the frontal surface of the head beneath the so-called 'fontanel,' which is defined by Brues ('15) as "a small, depressed pale spot on the front of the head between the eyes (isoptera)."

In surface views of the heads of the castes of *L. flavipes*, (figs. 8 to 12) the frontal gland appears as a rather triangular or rounded mass lying in the space between the two mushroom bodies. In the adult sexual forms and in the soldier a tiny round

opening may be observed in the cuticula above the anterior end of the gland, but I have not been able to distinguish any such opening in the worker or in the two nymphs. The opening is situated in a shallow depression of the surface, the fontanel, which is paler than the surrounding skin.

The frontal gland differs greatly in size in the various castes. It is smallest of all in the worker (fig. 11) and greatly elongated in the soldier (fig. 12). In neither of these castes does the frontal gland completely fill the space between the mushroom bodies. In the sexual forms the true adult (fig. 9) has the largest frontal gland; the nymph with long wing pads (fig. 8) has a gland of similar proportions but smaller; the nymphs with short wing pads (fig. 10) has an even smaller gland. In all the sexual forms the frontal gland completely fills the space between the mushroom bodies.

Looking down into the frontal gland as it is seen in surface views is like looking into a shallow crater. The edges rise into folds which approach each other and nearly meet at the antero-ventral end and enclose the central lumen. Sections show that the marginal cells of the gland are directly continuous with the hypodermal cells and that the cuticula above the center of the gland is slightly depressed and thin, consisting only of the primary or outer cuticula, the secondary, inner, cuticula ending at the gland margin. This depression and thinning of the cuticula is the structural explanation of the pale 'fontanel' spot seen on the outer surface of the head.

In the series of sections seen in figures 13 to 20, the first indication of the frontal gland is seen in a group of elongated hypodermal cells (fig. 13, *f.g.*) lying in the median line directly beneath the cuticula, which is here of normal thickness. This group of cells forms the anterior margin of the frontal gland and it should be noted that it occurs in the same frontal section as the simple eyes, or ocelli, *oc.*, a point which will be further discussed later. Figure 14, which is six sections farther back in the series, includes a section of the anterior part of the frontal gland, *f.g.* The high columnar cells of the gland are continuous with the low cuboid hypodermal cells; the cuticula is slightly depressed and

the secondary, inner, layer is lacking above the lumen of the gland. In figures 15, 16, 17, the deeper, central, part of the gland is shown; figures 18, 19, contain the posterior outpocketing of the gland, not connected with the margin in the figures, and since these sections are considerably farther back in the series, in the longest part of the head, the cuticula and hypodermal

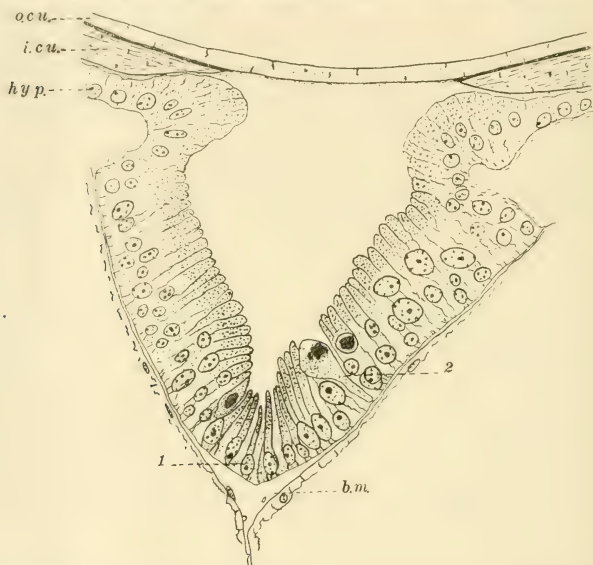


Fig. 5 Frontal section of the frontal or fontanel gland of a nymph with long wing pads. *B.m.*, basement membrane; *hyp.*, hypodermis; *i.cu.*, inner cuticula; *ocu.*, outer cuticula; *1*, long slender cells, probably not yet secretory in function; *2*, swollen cells, probably glandular. Homog. immers. 1.8 mm., oc. 6, reduced one fifth.

cells are now some distance from the brain, and, for considerations of space, are no longer included in the figures. Figure 20 shows the posterior wall of the frontal gland which ends in the next section.

a. The finer structure of the frontal gland. A closer study with the immersion lens shows that the frontal gland is composed of

the following parts, which are present, with slight modifications, in all the castes: (1) the epithelial cells, (2) the basement membrane, (3) muscle fibers attached to the basement membrane, (4) the fontanel nerve, an unpaired nerve running from the frontal gland to the protocerebral lobes, and here described for the first time.

1. Nymph with long wing pads. The epithelial cells (fig. 5, 1, 2) are of two kinds, of which the first far outnumber the second: 1, long slender columnar cells, with rounded distal ends, the distal cytoplasm taking a slightly deeper stain than the proximal portion, and the large oval nucleus situated near the base, 2, larger swollen cells whose cytoplasm contains vacuoles and irregular masses of a deeply staining substance. The nuclei of these cells are evidently basal but they are so crowded by the adjacent cells that I have been unable to distinguish them.

It will be noted that the lateral cells are more closely crowded together than those on the floor of the gland, in the median line, where the outlines of several cells may be clearly traced. It will also be noted that the marginal cells of the gland are lower and are directly continuous with the hypodermal cells. Mitotic figures are present here and there among the cells of the frontal gland in both nymphs, but no cuticula on the distal surfaces of the cells and no extracellular secretion have been observed. The only cells which appear capable of producing a secretion in the frontal gland of the nymph with long wing pads are the few scattered swollen cells marked 2, and these seem to have not yet discharged their secretion. This and the fact that there is no external opening may indicate that the frontal gland is not yet functional in this nymph.

The basement membrane (fig. 5, *b.m.*) upon which the epithelial cells of the frontal gland rest, is continuous with that of the hypodermal cells and is considerably thickened along the lower, ventral, surface. This membrane is composed of mesenchym cells and fibers and is beset with many irregular openings. In some specimens it is very delicate but thick and firm in others, and takes a deep pink stain with eosin, yellow with orange G. The ventral surface of the basement membrane near the posterior

end of the frontal gland is prolonged into a slender median process that passes downward posterior to the protocerebrum and connects the frontal gland with the great tentorial membrane that divides the interior of the head into the two parts in which lie the supra- and the subesophageal ganglia, and through which the ventral connectives pass. The basement membrane is similar in structure in all the castes.

Muscle fibers may be seen attached to the posterior or caudal surface of the basement membrane in the true adult, in surface views, but I have not observed them in either of the nymphs; muscle fibers are also attached to the basement membrane in the soldier and in the worker.

The fontanel nerve (figs. 2, 19, 23, 26, *f.n.*) is the slender unpaired nerve which makes its exit through apertures of the median ventral process of the basement membrane of the frontal gland, and which passes vertically downward and enters the median dorsal surface of the protocerebral lobes at a definite point, namely: between the large posterior roots of the mushroom bodies and in the same frontal plane in which the ocellar nerves enter the protocerebral fibrous core. This is the first time that this nerve has been described or figured in any termite brain. It is present in a similar position in all the castes and phases of *L. flavipes* that have been examined, and it is undoubtedly a strand of nerve fibers, although a very delicate one. I have provisionally termed this nerve the fontanel nerve, and we shall return to it again after a discussion of the frontal gland in the other castes.

2. The true adult. I have not yet succeeded in making good sections of the true adult's frontal gland, but in a surface view of an entire brain and frontal gland dissected out and mounted it is most evident that the inner surface of the frontal gland is lined with a wavy chitinous cuticula, the channels of which converge toward the anterior opening on the surface of the head.

Although I have unfortunately not studied the true adult frontal gland in sections and can not therefore describe the epithelial cells, the facts that it is larger than in the nymph with long wing pads, and that it has acquired a chitinous cuticula,

and an opening to the exterior, seem to indicate that this gland probably produces secretion and is therefore functional. The true adult frontal gland in this respect would represent a later phase in development than that of the nymph with long wing pads.

3. The nymph with short wing pads. The frontal gland of this nymph is similar in all respects except size to that of the nymph with long wing pads. Although this gland as a whole is smaller (fig. 6, *A*), its component epithelial cells are similar in height and width to those of the gland just ascribed. The two types of cells, the numerous long slender ones, 1, and the few swollen gland (?) cells, 2, are also present. No cuticula is dis-

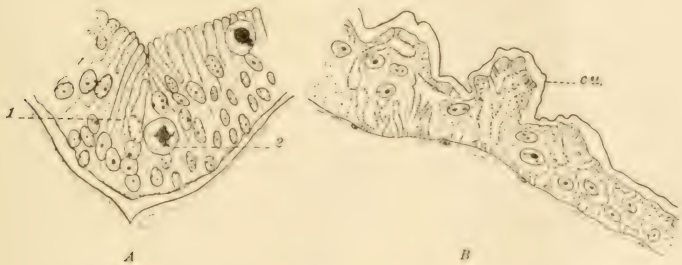


Fig. 6 Sections of the frontal gland. *A*, the median part of the gland of a nymph with short wing pads; *B*, the lateral part of the gland of a soldier. Homog. immers. 1.8 mm., oc. 6, reduced one fifth.

tinguishable on these cells and there is no external opening. This gland is evidently not yet functional.

4. The soldier. The cells of the elongated frontal gland of the soldier form a continuous synectium, a part of which is shown in section in figure 6, *B*. The synectium rests upon a thin basement membrane, the nuclei are rather widely separated, and the distal, inner, surface is bordered by a wavy glassy and porous cuticula. The entire inner surface is thrown into folds but the average height of the component cells is lower in the soldier than in the nymphs. The cells are all of one kind, and are evidently glandular in function, the abundant cytoplasm contain-

ing a network of intracellular canals that open into the lumen of the gland by pores in the cuticula. In surface views of the soldier head strands of muscle fibers seem to be connected with the lateral and posterior surfaces of the frontal gland, and, as was stated above, an opening in the cuticula of the head above the gland may be clearly seen. Although no secretion was

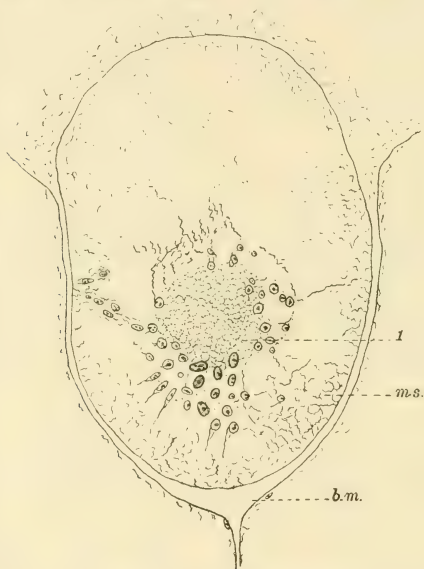


Fig. 7 Frontal section of the frontal gland of a worker. *B.m.*, basement membrane; *ms.*, mesenchyme; *I*, epithelial cells. Homog. immers. 1.8 mm., oc. 6, reduced one-fifth.

actually observed in the lumen of the gland, the intracellular canals and the pores in the glandular cuticula seem to indicate that the frontal gland in the soldier is in active secretion.

5. The worker. The frontal gland of the worker as seen in surface view (fig. 11) is very small and is surrounded by an empty space. Sections (figs. 7, 25) show that this gland, although bearing a morphological resemblance to that of the

nymphs of the sexual forms, is in a less highly developed condition, a condition that seems to be secondarily, indeed regressively, modified, rather than primitive. In the area between the mushroom bodies we find (fig. 7) a cup formed by basement membrane, *b.m.*, which, although smaller, is somewhat similar in outline to that of the two nymphs just described. The space within the basement membrane is not, however, entirely filled by the cells of the frontal gland. The cells, *I*, occupy only the central part of the cup, the remainder consists of a membranous network of mesenchym, *ms.*, similar to and indeed continuous with the tentorial membrane which lies posterior to the frontal gland and to the supraesophageal ganglion in all castes. The cells of the frontal gland as shown in figure 7, *I*, are cut in a plane parallel to their distal upper surfaces, the cell bodies polygonal in outline and closely pressed together, and only a few nuclei are present. In the following sections from deeper portions of the gland, many more nuclei occur. The cells are all of the slender elongated type, but much smaller in every dimension than the slender elongated cells of the two nymphs above described. None of the swollen secretory cells are present. From the general appearance it may be inferred that this gland in the worker is not only nonfunctional but degenerate in structure.

b. General discussion of the frontal gland. To summarize the different structural and functional conditions of the epithelial cells of the frontal gland in the various forms of *L. flavipes*, we find that in the soldier the frontal gland is evidently functional and in active secretion. An external opening is present and the epithelial cells are all glandular, united in a syncytium containing a network of intracellular canals and covered by a porous cuticula.

In the true adult the frontal gland is probably functional. An external opening is present above the gland; the gland has enlarged since the last nymphal phase, and a cuticula resembling that of the soldier is present upon the inner surface of the epithelial cells. No statement as to the cells themselves can be made now, since no sections of this gland have been studied.

In the two nymphs with long and short wing pads the frontal gland seems not yet functional. There is no external opening above the gland; the cells which appear capable of producing secretion are few and scattered, while most of the cells are narrow and slender with no evidence of any contained secretion, but resembling rather sensory cells in their form and contents. The last nymphal molt will remove the outer cuticula, and the absence of the inner cuticula, described above, will produce the external opening seen in the true adult. At the same time the epithelial cells which were not glandular in the nymphal phases may form a cuticula on their inner surface, acquire the power of secretion, and become fully glandular in function in the adult condition. It would therefore seem that the frontal gland of nymphs with long and short wing pads represents an earlier phase of development than that of the true adult or soldier. On the other hand, it is apparent that the frontal gland of the worker is in a secondarily modified and regressive condition. It is evidently nonfunctional, the cells showing no signs of secretion; it is also degenerate in structure, for the cells, although of the same form as in the nymphs, are much smaller, are numerically fewer, and are partly replaced by a network of mesenchym.

The study of the frontal gland might end here with the frequently asked and still unanswered question: What is the function of the frontal gland? The question, however, that has presented itself most forcibly throughout my study of this organ is, Why should most of the epithelial cells of the frontal gland in the nymphal phases bear such a striking resemblance to sensory cells? Young gland cells may usually be recognized as such even in their early phases, and they are rarely so slender and elongated. If, as the biogenetic law teaches us, ancestral structure is frequently to be observed in young, that is, in developing organs, is this then an instance? It is possible that the frontal gland of the termite, whose function is unknown and whose structure needs further investigation, may represent an ancestral organ, highly developed and secondarily modified in some individuals and vestigial in others, but whose primitive

structure and perhaps even whose origin may be revealed in the developing nymphal stages.

This supposition leads naturally to the query, What then was the frontal gland if it were originally not a gland? In answer I wish to offer the suggestion that the frontal gland of the termites may be a modification of the ancestral frontal ocellus or simple eye that is found in many insects, and of which Berlese ('09) says that if the third eye is not present it should be considered as having disappeared. "Ordinamente sono in numero di tre e giacciono sul vertice o sulla fronte—. . . . In taluni casi se ne osservano solo due (Grillidae ecc.) ma il terzo deve essere considerato come abortito."

Several naturalists of the nineteenth century, notably Joly ('49) and Lespès ('56), in their study of termites observed the pale depressed spot now known as the fontanel and described it as the third, median, ocellus.

Hagen ('55-'60) in his description of *Termes flavipes*, Kollar, states that there is "Mitten auf dem Scheitel ein flachen Eindruck mit einem erhabenen helleren Fontanellepunkt." Referring to the earlier view that this 'fontanel point' was a third ocellus, he writes:

Ein drittes Nebenauge fehlt bestimmt; dass das so oft dafür angegebene Organ (dem übrigens stets eine gewölbte Hornhaut fehlt) kein Nebenauge sein kann, hatte man schon aus seiner Lage abnehmen können. Es liegt nämlich immer beträchtlich höher auf dem Scheitel als die Nebenaugen, ein Verhältniss, dass bei den Insekten ohne Beispiel ist; das dritte Nebenauge liegt bei allen bekannten Insekten näher dem Munde, als die seitlichen.

Nassonoff ('93) described the structure of the frontal gland in a termite soldier.

Czerwinsky ('97) studied a number of termites, including some tropical forms. He described the structure of the frontal gland as follows:

Die Stirndrüse liegt hinter dem Oberschlundganglion ein wenig über demselben und gehört immer zu den mehrzelligen Drüsen, in der Bildung aber stellt sie mannigfaltige Grade der Entwicklung dar. In dem einfachen Fälle besteht sie aus einer Schicht in die Länge verzogener Hypodermiszellen. Solchen einfachen Bau findet man bei vielen

Arbeitern (*Eutermes* sp., *Eutermes Ripperti*, *Termes Mülleri*). Einen komplizierteren Bau finden wir bei einer anderen Gruppe der Arbeiter (*Termes lucifugus*, *Eutermes capricornis*, *Termes dirus*) und bei den geflügelten Insekten. Die Drüsenzellen sind hier stark in der Länge ausgezogen und bilden zusammen ein Säckchen. Das Sekret der Drüsenzellen häuft sich zwischen denselben und dem Chitin und gelangt wahrscheinlich erst durch die Körperoberfläche nach aussen. Das Chitin ist hier sehr dünn und bildet ausserhalb einen weissen Fleck (Fontanelpunkt der Beschreibungen). Dieser Fontanelpunkt wurde sogar von einigen Verfassern als ein drittes Nebenauge angenommen.

Hagen leugnet die Existenz des dritten Nebenauges, aber erklärt nicht die Bedeutung des Fontanelpunktes. Der Fontanelpunkt mit der anliegenden Oberfläche bildet eine wenig deutliche Vertiefung. Durch tiefere Einsenkung der Drüsenzellen ins Innere mit der darüberliegenden Cuticula kann sich ein Reservoir bilden aus dem das Sekret durch eine Oeffnung austritt. Eine solche Bildung stellt die Stirndrüse bei den Soldaten dar. Den kompliziertesten Bau der Stirndrüse finden wir bei den soldaten Nasuti und Arbeitern Nasuti, bei denen ausser dem schon beschriebenen Bau ein Ausführsgang in der Nase vorkommt.

Holmgren, '09, finds the fontanel gland similar in structure in the imago and worker of *Eutermes chaquimayensis*. The glands consist of a single layer of elongated cells. Two muscles, the mm. retractores fontanellae, are attached near the tip of the gland. No actual secretion was observed. In the soldier, the frontal gland consists of two clearly defined parts, (1) the glandular part, of high cylindrical cells, with a row of short rods (Stäbchen) on the distal ends, (2) the duct of low and culcated cells.

Feytaud ('12), in a study of 'Le termite lucifuge,' writes:

La glande fontanelle ou glande frontale est formée par la différenciation d'une groupe de cellules hypodermiques au niveau de la partie supérieure du front, au-dessous de la zone plus claire désignée sous le nom de fontanelle. Dans beaucoup de cas, la glande frontale n'est représentée que par une différenciation des cellules hypodermiques, sans dépression. Enfin, dans certaines formes, elle est complètement absente.

Feytaud cites the nymph of the second form as an instance of a slightly differentiated frontal gland, stating that like that of the worker the frontal gland in this nymph is merely a zone of elongated hypodermal cells. In the conclusion of this paper Feytaud asks "What is the rôle of the frontal gland?"

Wasmann ('02), in describing the new genus *Speculitermes* states that it is "Ausgezeichnet durch die grosse, unpaare Stirnocelle, welche nicht nur bei der Imago, sondern auch beim Arbeiter vorhanden ist."

Now, although I am firmly convinced that the fontanel spot with its opening is not an ocellus or simple eye, I feel that there is considerable evidence that the frontal gland of the termites may have developed phylogenetically from an ancestral median eye and its ocellar nerve. In other words, the frontal gland may have first arisen as a modification of the hypodermis at the point where the ancestral median eye was formerly situated, and then may have extended inward into the head along the course of the former median ocellar nerve, the proximal part of which may still persist in the nerve which I have termed provisionally the fontanel nerve.

The evidence in support of this view, gained from the study of the different forms of *L. flavipes*, will now be presented.

1. The anterior surface of the frontal gland of the nymphs and adults of the sexual forms lies in the same frontal plane as the lateral ocelli. The posterior end of the frontal gland lies in the region in which the lateral ocellar nerves enter the protocerebral fibrous core. The frontal gland has therefore the same linear extent as the lateral ocelli and the ocellar nerves.

2. The nerves from the lateral ocelli run from the ocelli in toward the median line on the outside of the brain sheath, directly beneath the hypodermis, then piercing the brain sheath, they run downward and backward within the nerve cell layer of the brain, finally entering the fibrous core of the protocerebral lobes at a definite point, namely: immediately behind the posterior dorsal commissure. The fontanel nerve from the frontal gland enters the protocerebral fibrous core in the median line, immediately behind the posterior dorsal commissure, and, according to the present theory, may represent the median ocellar nerve (fig. 19).

3. The lateral ocelli are derived from the hypodermis, and the tips of the visual cells still lie in contact with and perhaps between the hypodermal cells. The frontal gland is directly con-

tinuous with the hypodermis, and its cells are modified hypodermal cells.

4. The inner cuticula is thinner at the margins and lacking above the center of the lateral ocelli (fig. 13). The same is true of the inner cuticula above the frontal gland (fig. 16).

5. The cells of the frontal gland in the adult stages are secretory in function and resemble other gland cells in structure, but in the developing nymphal stages the cells produce no secretion and bear a striking resemblance to the slender elongated visual cells found in insect ocelli, Hesse ('01 b). According to our theory this ontogenetic phase corresponds to a primitive, phylogenetic condition. In this connection it is significant that in very young nymphs the frontal gland is barely distinguishable in surface views, while the brain and eyes are quite well developed. The further study of these youngest nymphs by means of sections may give additional evidence.

6. The phylogeny of the termites upon the basis of the morphology of the frontal gland suggested by Holmgren ('09) may be referred to as additional evidence. Holmgren has divided all adult sexual termites into three groups: The first group, including all the higher termites, consists of those forms with a sack-like frontal gland (sackförmige Drüse) and a fontanel opening; the second group, including part of the lower termites, consists of those forms with a plate-like nonglandular 'gland' (Fontanellplatte) and no fontanel opening; the third group, including the remainder of the lower termites, consists of forms in which both the plate-like nonglandular 'gland' (Fontanellplatte) and the fontanel opening are lacking. Holmgren believes that the sack-like fontanel gland and the nonglandular plate are morphologically equivalent, and that both are derived from a common ancestor without either gland or plate. He further remarks that no fontanel is found in the Blattidae, which in this respect are more nearly related to the lower termites. To quote his exact words:

Versuchen wir nun eine Phylogenie der Termiten auf dem Verhalten der Fontanelle zu gründen, so möchte erstens bemerkt werden, dass diejenigen Formen, welche Fontanelldrüsen (Fontanellplatte und sack-

förmige Drüse) besitzen, aus Formen ohne drüsige Fontanelplatte stammen müssen. Da es wenig wahrscheinlich ist, dass eine drüsige Fontanellgewebe unabhängig bei verschiedenen Termitengruppen entstanden ist, so müssen wir diejenigen mit Fontanelplatte und diejenigen mit schlauchförmiger Fontanelldrüse von einer gemeinsamen Ausgangspunkt ableiten.

7. It is always questionable whether comparisons should be drawn between invertebrates and vertebrates, but it does not seem wholly improbable that the frontal gland of the termites may have had a phylogenetic history similar to that of the vertebrate pineal gland. The disappearance of the simple median vertebrate eye may be understood in conjunction with the increasing complexity of the compound eyes, and a brief survey of termite habits may throw light upon the causes of the disappearance of this median invertebrate eye. The true sexual adults are the most conservative and primitive in structure and in habits of all the castes. They are deeply pigmented, possess long functional wings, and for a short time lead an aerial existence like other typical winged insects, and unlike all the other members of the community. After the short period in the air, which termite observers tell us is not a nuptial flight, the true adults abandon their normal ancestral mode of life and begin a secondarily acquired existence within the total darkness of their burrows. Here the long wings are shed, mating takes place, and from henceforth they live within the dark and narrow chambers of the nest. It is possible that this sudden and marked change of environment may account in part for the disappearance of the median eye in the ancestral termite and that the frontal gland may have arisen in response to some new need.

SUMMARY OF RESULTS

The purpose of this study is to compare the brains of the different castes of *L. flavipes*, and, further, to compare them with those of the castes of ants, since both termites and ants are social insects with a highly organized community life, but with a very different degree of specialization and intelligence.

The forms whose brains are here discussed are: the nymph of the first form, with long wing pads, the nymph of the second

form, with short wing pads, the soldier, the worker, and, to some extent, the true adult.

1. There is no differentiation between the brains of the males and females of any caste or stage of *L. flavipes*.

2. There is very little differentiation between the brains of the different castes and stages of *L. flavipes* here discussed.

a. The most marked differentiation is in the optic apparatus, a correlation existing between the degree of development of the compound eyes and the size of the optic lobes. The latter are large in the nymphs and adults of the sexual forms, but are greatly reduced in the worker and soldier.

b. The mushroom bodies differ very little in size, by actual measurement and in the estimated number of cells. They are largest in the worker, smallest in the soldier, and are intermediate in size in the sexual forms, although the mushroom bodies of the true adult are nearly as large as those of the worker.

c. The antennary lobes are very similar in size, but are largest in the worker and in the sexual forms and slightly smaller in the soldier.

3. The mushroom body stalks do not end beneath the central body, as was formerly thought, but divide into three roots, the anterior, central body, and posterior roots. The latter are expanded into large and prominent lobes.

4. The labral nerves arise from the ventral surface of the frontal ganglion, and not directly from the labrofrontal nerves.

5. The termite brain as a whole is very similar in structure to the brain of ants, with the notable exception of the mushroom bodies which are of a much more simple and primitive type. This is apparent in: (a) the nerve cells, which are all very small and of equal size, (b) the separation of these cells into three groups, instead of the four of ants, (c) the incomplete separation of the two lobes.

Only the two lateral simple eyes or ocelli are present in the nymphs and adults of the sexual forms, and none are found in the worker or soldier. These ocelli are very simple and primitive in structure, without lens or pigment, and the ocellar nerves do not expand into ocellar lobes as in ants.

6. The frontal gland is a gland found in all the castes of *L. flavipes*, situated on the postero-dorsal surface of the brain in the space between the mushroom bodies. The gland is composed of epithelial cells which are continuous with the hypodermal cells. A nerve, termed provisionally the fontanel nerve, runs from the frontal gland into the brain.

In the true adult and soldier the frontal gland is evidently functional; in the worker it is nonfunctional and degenerate in structure; in the nymphs with long and short wing pads it is evidently not yet functional, a few cells are glandular in appearance with contained secretion, the large majority of the cells are slender and elongated and resemble sensory, visual, cells.

7. The suggestion is made that the frontal gland may have arisen phylogenetically from the ancestral median ocellus which is now lacking in the termites, and that the 'fontanel' nerve may be a vestige of the former median ocellar nerve.

The arguments for this view are based upon the position and the structural resemblances of the frontal gland and the lateral ocelli, upon the presence of the 'fontanel' nerve in the same frontal section in which the lateral ocellar nerves enter the brain, upon the resemblance of the frontal gland cells of the developing nymphal phases to visual cells, and upon the facts collected by Holmgren regarding a phylogeny of the termites based upon the morphology of the frontal gland.

WELLESLEY, MASS.

AUGUST 18, 1916

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⁶ More extensive bibliographies of the termites may be found in the works of Holmgren ('11), Feytaud ('12), and Snyder ('15).

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PLATE 1

EXPLANATION OF FIGURES

8 and 9 are drawn from whole mounts of the heads. Figure 10 is a combination drawing of the outline of the head and the brain, the latter added from a dissected and mounted brain. The stippling represents the nerve cell layer, the fibrous matter is blank. Obj. 16, oc. 6, stage level, reduced one-third.

8 The head and brain of a nymph with long wing pads.

9 The head and brain of a true adult.

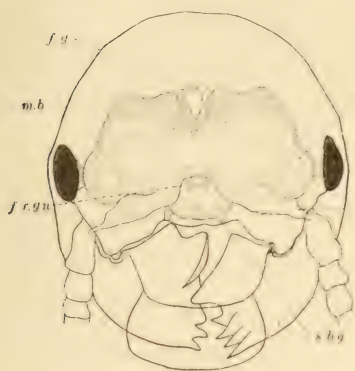
10 The head and brain of a nymph with short wing pads.

EXPLANATION OF PLATES

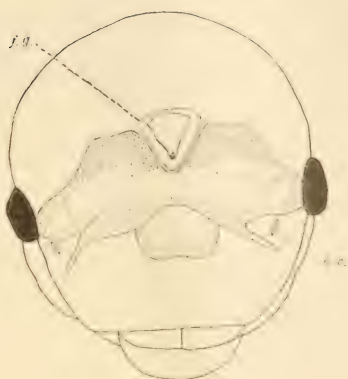
All figures are drawn with the Spencer camera lucida and with Spencer lenses

ABBREVIATIONS

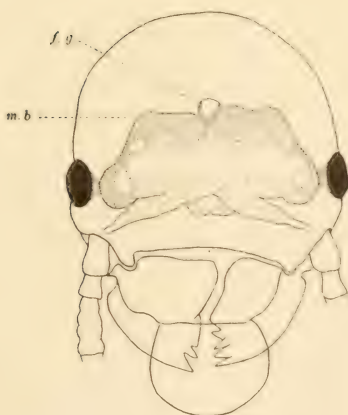
<i>a.cm.</i> , anterior dorsal commissure	<i>m.b.s.</i> , mushroom body stalk
<i>a.l.</i> , antennary lobe	<i>md.n.</i> , mandibular nerve
<i>a.r.m.b.</i> , anterior root of mushroom body	<i>m.f.</i> , middle fiber mass
<i>b.m.</i> , basement membrane	<i>ms.</i> , mesenchym
<i>c.b.</i> , central body	<i>mx.n.</i> , maxillary nerve
<i>c.b.r.</i> , central body root	<i>oc.</i> , ocellus
<i>c.e.</i> , compound eye	<i>oc.n.</i> , ocellar nerve
<i>cu.</i> , cuticula	<i>o.cu.</i> , outer cuticula
<i>f.g.</i> , frontal gland	<i>oe.</i> , oesophagus
<i>f.n.</i> , fontanel nerve	<i>o.f.</i> , outer fiber mass
<i>fr.gn.</i> , frontal ganglion	<i>o.l.</i> , optic lobe
<i>gl.</i> , glia cells	<i>p.l.</i> , protocerebral lobe
<i>hyp.</i> , hypodermis	<i>p.r.m.b.</i> , posterior root of mushroom body
<i>i.cu.</i> , inner cuticula	<i>r.n.</i> , recurrent nerve
<i>i.f.</i> , inner fiber mass	<i>sb.g.</i> , subesophageal ganglion
<i>la.n.</i> , labral nerve	<i>tr.cm.</i> , tritocerebral commissure
<i>lb.n.</i> , labial nerve	<i>tr.l.</i> , tritocerebral lobe
<i>l.f.n.</i> , labrofrontal nerve	<i>v.c.</i> , ventral connective
<i>m.b.</i> , mushroom body	



8



9



10

PLATE 2

EXPLANATION OF FIGURES

11 and 12 are drawn from whole mounts of the head. The stippling represents the nerve cell layer, the fibrous matter is blank. Obj. 16, oc. 6, stage level, reduced one third.

11 The head and brain of the worker.

12 The head and brain of the soldier.

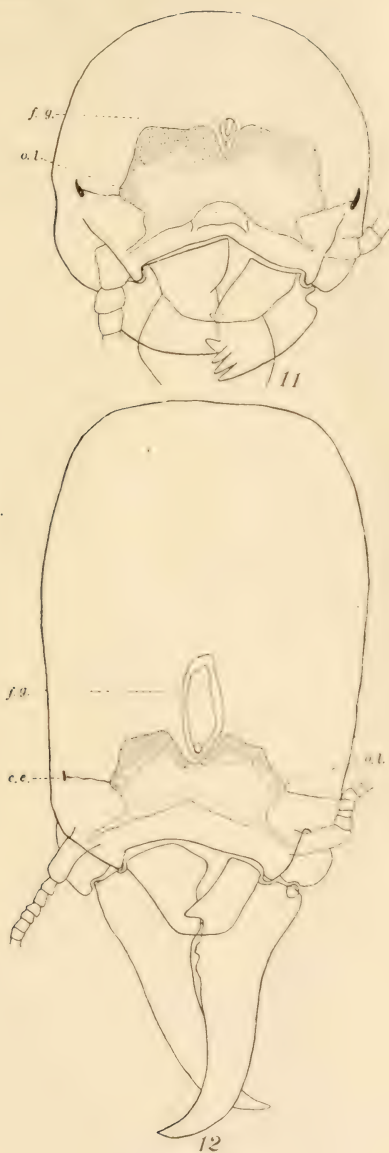


PLATE 3

EXPLANATION OF FIGURES

13 to 20 are taken from a series of frontal sections of the head of a male nymph with long wing pads, beginning at the frontal surface. The fibrous core is mottled, the nerve cell layer is blank. Obj. 16, oc. 6, table level.

13 Section through the anterior part of the protocerebral lobes, *p.l.*, showing the lateral ocelli, *oc.*, and the anterior margin of the frontal gland, *f.g.*

14 Section showing the connection of the anterior roots, *a.r.m.b.*, and the stalks, *m.b.s.*, of the mushroom bodies.

15 Section through the central body, *c.b.*, and the central part of the mushroom bodies, *m.b.*

16 Section showing the entrance of the central body roots, *c.b.r.*, into the central body.

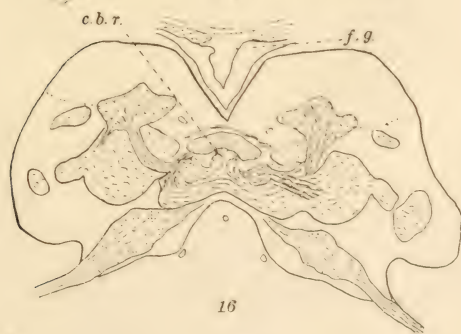
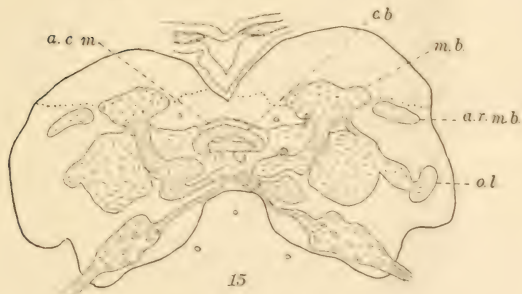
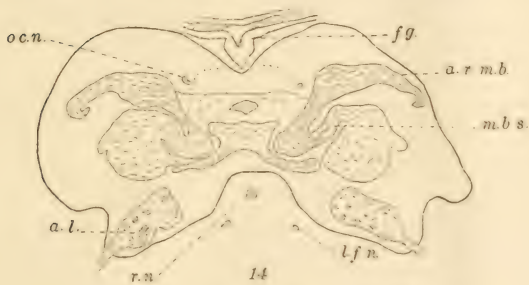
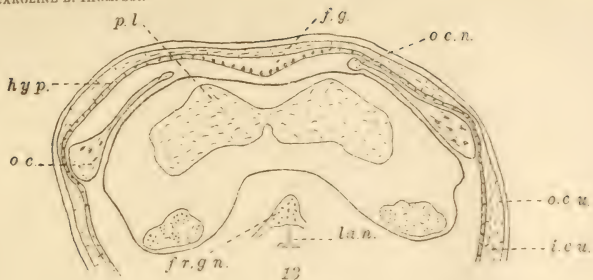


PLATE 4

EXPLANATION OF FIGURES

17 Section through the posterior part of the central body, showing the origin of the posterior roots of the mushroom bodies, *p.r.m.b.*

18 Section showing the posterior dorsal commissure, *p.cm.* The exit of the labrofrontal nerve, *l.f.n.*, from the nerve cell layer is also shown.

19 Section showing the entrance of the ocellar nerves, *oc.n.*, and of the fontanel nerve, *f.n.*, into the protocerebral fibrous core. The large lobes of the posterior roots of the mushroom bodies, *p.r.m.b.*, are very prominent, and are also connected with the protocerebral fibrous core.

20 Section showing the connection between the protocerebrum, deutocerebrum, and tritocerebrum.

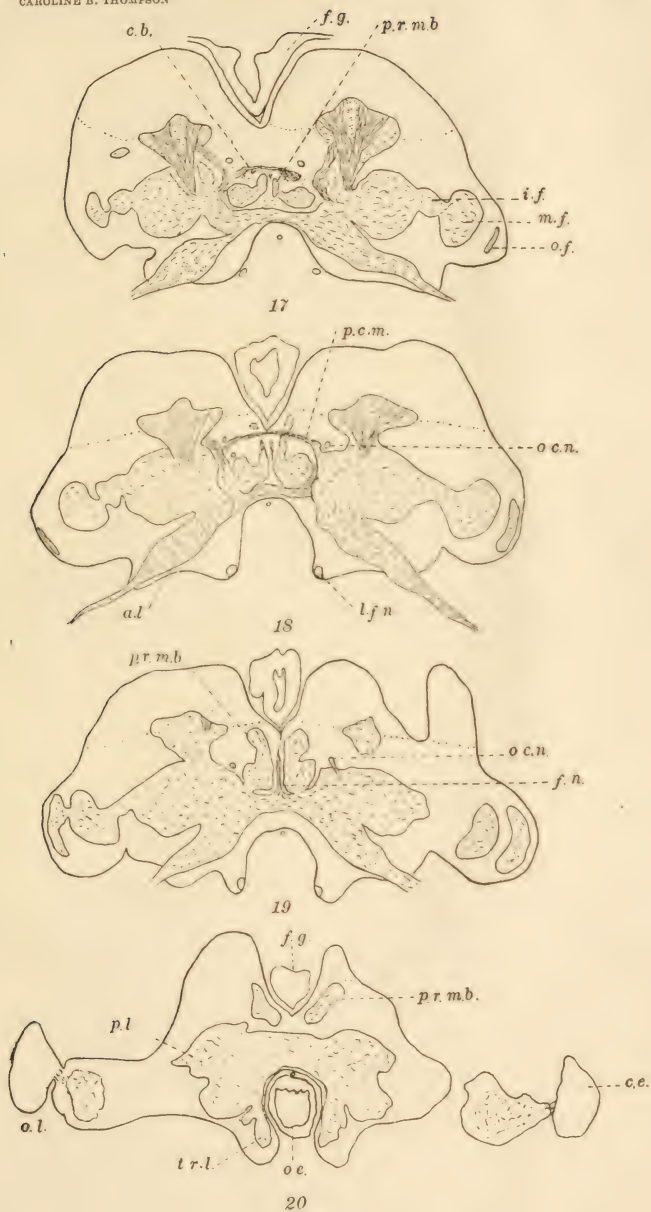


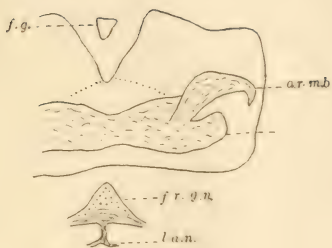
PLATE 5

EXPLANATION OF FIGURES

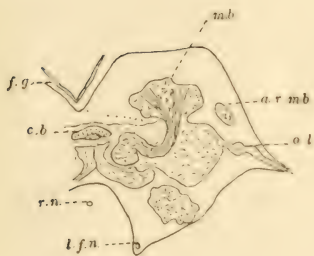
- 21 to 26, obj. 16, oc. 6, table level.
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- 22 Frontal section through the central part of the brain of a soldier.
- 23 Frontal section through the posterior part of the brain of a soldier.
- 24 Frontal section through the anterior part of the brain of a worker.
- 25 Frontal section through the central part of the brain of a worker.
- 26 Frontal section through the posterior part of the brain of a worker.



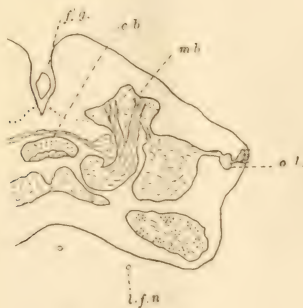
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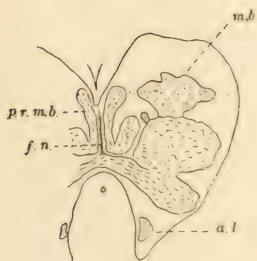
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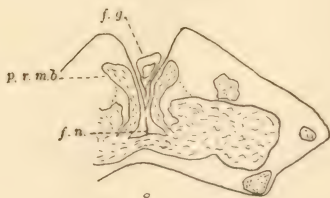
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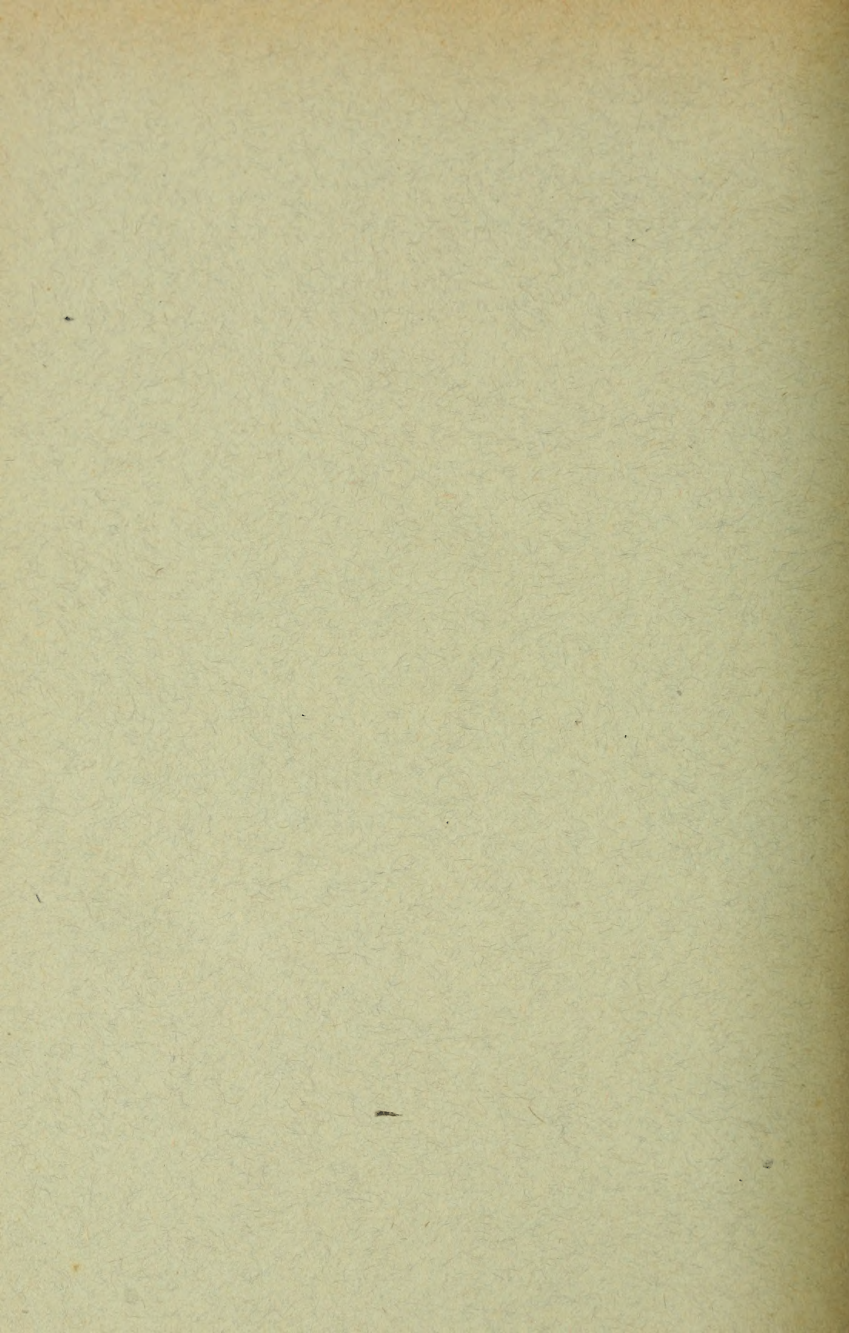
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